Endurance performance: the integrative physiology of resisting fatigue

Yolande Xanthe Rocille Harley

Thesis Presented for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Human Biology,

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

September 2004

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

University of Cape Town

Doctoral Degrees Board
University of Cape Town
Private Bag Rondebosch
7701 South Africa
Tel: (021) 650-2155
Fax: (021) 650-2138
vnaidoo@bremner.uct.ac.za

Declaration

PhD THESIS TITLE:

Endurance performance: the integrative physiology of resisting fatigue

I, Yolande Harley

hereby

(a) grant the University of Cape Town free licence to reproduce the above thesis in whole or in part, for the purpose of research;

(b) declare that:

(i) the above thesis is my own unaided work, both in concept and execution, and that apart from the normal guidance from my supervisor, I have received no assistance except as stated below:

(ii) neither the substance nor any part of the above thesis has been submitted in the past, or is being, or is to be submitted for a degree at this University or at any other university except as stated below

I am now presenting the thesis for examination for the degree of PhD.

SIGNED: signature removed

DATE: 23/09/2004

Table of Contents

Abstract

OBJECTIVE

Fatigue is a ubiquitous phenomenon that can affect people's performance and quality of life. While known in the exercise sciences as a limiting factor for athletic performance, fatigue during physical activity is a much farther-reaching phenomenon that affects people from all cultures, all career paths and all levels of health. Fatigue has large-scale socioeconomic ramifications, and research into the causes of, and possible counter-measures to attenuate symptoms of fatigue is therefore worthwhile from a health, a sporting and an economic perspective.

An elegant approach to studying fatigue is to use the model of the endurance athlete, a healthy individual who regularly strives to overcome fatigue. As such, fatigue during endurance activity is a popular area of study in the exercise sciences. Despite extensive research in this area, there is considerable disagreement as to exactly what factors are involved in fatigue during physical activity, or what limits endurance performance. Even the definition of fatigue is still controversial. Fatigue can be viewed as a form of physical failure, as a symptom reported by the exercising individual, or as an entity that consists of the various physiological processes occurring during endurance activity. Fatigue can also be regarded as a sensation that reflects the conscious awareness of physiological changes, or even as a sensation that functions as an active regulator of exercise intensity, instead of simply being a passive consequence of physiological processes. As such, fatigue can play both a negative role during exercise, by limiting physical performance, and a positive role, by preventing the exercising individual from permanently harming their body with excessively stressful activity.

Much of exercise physiology research has used a reductionist approach to investigating fatigue during physical activity, by examining individual variables or systems in the body. While there is considerable scientific value to this form of research, it can be misleading when the findings are not observed in the context of the body as a whole. Many studies have attempted to describe a single physiological system as the ultimate cause of

fatigue, and have sought to identify which factor in this system is the limiting one. However, it is likely that fatigue involves many different, but interrelated physiological factors and processes, and consequently research examining multiple physiological systems using an integrated approach is needed.

Therefore, the objective of this thesis was to assess the hypothesis that any single physiological factor or system can wholly account for fatigue, by examining whether fatigue instead involves numerous factors from different physiological systems in the body. The number of factors in each physiological system that could be involved in fatigue is vast, and investigating all of these factors is beyond the scope of this thesis. Consequently, this work has not comprehensively investigated each of these physiological systems, but rather highlighted aspects from each system that may play a role in fatigue in order to illustrate how varying factors may function in an integrated manner during fatigue. This thesis therefore addressed the putative multifaceted and integrative nature of fatigue by investigating its association with multiple parts of human physiology during endurance performance. Endurance performance has been used as a proxy for fatigue resistance, seeing as these two factors can be regarded as analogous, in practical terms, and due to fatigue itself not technically consisting of anything measurable. Hence, the title of this thesis: "Endurance performance: the integrative physiology of resisting fatigue".

METHODS

The importance of different physiological systems in fatigue during physical activity was examined by investigating the relationship between endurance performance and physiological factors from the cardiorespiratory, intramuscular, neuromuscular and central nervous system. A group of 32 distance runners, whose personal best times for a 10 km road race ranged from 30 to 41 min, were used as subjects when investigating the cardiorespiratory, intramuscular and neuromuscular factors. In addition to examining the potential relationships between physiological traits and endurance performance in distance runners in general, the measured physiological variables were also compared between 16 black and 16 white South African distance runners, matched for 10 km personal best race time. The "black South African" runners were all males living in the Western Cape of South Africa, of Southern African descent, with the majority being

descended from the Xhosa tribe. The "White South African" runners were all Caucasian males living in the Western Cape of South Africa, of European descent (including English, Scottish, Irish, German and Dutch). Black South African runners are a population group specifically known to perform well in long distance running. Therefore, identifying which traits these runners have that are associated with superior endurance performance, and that are different to a 'control' group of white runners, may suggest which physiological factors can be important in endurance performance, and hence fatigue resistance, in general, ethnicity aside.

The cardiorespiratory variables that were investigated, using a series of exercise tests, were oxygen consumption, running economy, heart rate and respiratory exchange ratio. In addition the plasma concentrations of lactate, sodium and potassium were studied. Anthropometric variables were also assessed. Regression analyses were performed for these variables with the reported endurance performance measure, 10 km personal best running time, as well as with a laboratory-based measure of running performance, peak treadmill running velocity. These variables were then compared between the black and white South African runners in order to determine if differences in these variables between the two ethnic groups could be related to the superior endurance ability and fatigue resistance of the black South African runners. The intramuscular factors that were investigated included vastus lateralis muscle fibre composition and the muscle content of monocarboxylate transporters (MCT) 1 and 4. Similar to the cardiorespiratory factors, these variables were correlated with 10 km running time and compared between the two ethnic groups. In the examination of the neuromuscular system, muscle strength, muscle endurance and muscle elasticity, along with the neuromuscular recruitment patterns associated with these, were investigated. The tests performed therefore included maximal knee extension strength tests (isometric and isokinetic), maximal and submaximal isometric knee extension fatigue tests, stretch-shortening cycle jump tests, stride parameter measurements and electromyographic analysis of the knee extension and stretch-shortening cycle tests. These factors were again correlated with 10 km running time and compared between the black and white South African runners.

A different set of 19 subjects of a wide range of athletic ability were used to investigate the relationship between fatigue and the central nervous system, specifically the brain. This protocol was designed such that healthy people of any level of athletic ability could

complete it, and ethnic comparisons were not made, as the goal was to investigate what fatigue-related changes in the brain occur in different people, and not specifically athletes. Electroencephalographic activity, electromyographic activity and force tremor were measured during a submaximal isometric fatiguing knee extension, and the data analysed for the 19 subjects in order to examine how fatigue affects the brain in ways common to many different people.

Lastly, the cardiorespiratory, intramuscular, neuromuscular and central nervous system factors were re-examined in an integrative manner, making use of multiple regression analysis, with the putative multifaceted nature of fatigue in mind.

RESULTS AND DISCUSSION

Investigation of intramuscular, neuromuscular and central nervous factors revealed novel findings related to fatiguing endurance activity. Muscle MCT4 content was positively associated with 10 km running performance, possibly due to enhanced MCT4-mediated efflux of lactate and H^+ ions from the muscle cell during exercise. Stride length and stride length per height were also positively associated with running performance, possibly due to a function of the power or elasticity in the leg muscles. Similarly, performance during the maximal isometric fatiguing knee extension was also positively correlated with 10 km performance, suggesting that fatigue resistance during continuous, static exercise is associated with performance during dynamic exercise, at least in trained distance runners. Another novel discovery was that brain activity in the subject group changed significantly during fatiguing exercise in multiple frequency bands, over many areas of the brain and in both cortical hemispheres. Fatigue appears to be linked to multiple brain regions and processes, and it is proposed that a 'fatigue matrix' exists in the brain, encompassing the network of brain areas that is activated with fatigue.

In addition to these novel findings, the cardiorespiratory investigation confirmed previous reports of a relationship between endurance performance and maximal oxygen consumption, peak treadmill velocity, running economy and anthropometric variables associated with body fat.

There were several novel findings with regard to physiological and performance comparisons of black and white South African runners. The two ethnic groups had significantly different concentric quadriceps strength as well as different exercising plasma sodium and potassium levels. The black runners performed better than the white runners during sustained submaximal isometric knee extension, suggesting that the superior fatigue resistance of black South African runners is not restricted to running or even dynamic exercise. Electromyographic changes accompanying isometric fatigue suggested less peripheral fatigue in the black compared to the white runners, as did force output responses to muscle stimulation.

The efficiency of stretch-shortening cycle functioning was not different between the two ethnic groups, suggesting that it is not a difference in elastic energy utilisation that allows black South African runners to perform better than white runners. The black runners, however, covered more distance per stride for their height when running than the white runners did, indicating that a difference in biomechanics or muscle power could be related to the superior performance of black compared to white South African runners. This thesis confirmed previous reports of a difference in exercising plasma lactate concentrations between black and white South African runners, however showed that this is not likely to result from a difference in the total cellular density of MCT1 or MCT4 in the muscle, as this was not different between the ethnic groups. In this regard, an ethnic comparison of the expression of these MCT's in the mitochondrial and sarcolemmal fractions is recommended for future study.

In addition to the novel findings, many previously reported differences and similarities relating black and white South African runners were confirmed. These include similarities in maximal oxygen consumption and muscle fibre composition, but differences in running economy, isometric quadriceps strength, and anthropometrical variables relating to body size, and to body muscle and fat content.

Therefore, there were factors from each of the physiological systems investigated that were related to endurance performance and hence fatigue resistance, and many physiological factors were different between black and white South African runners. Multiple regression analysis also suggested that many factors are involved in endurance performance, including variables measured from the body at rest, and those that can be

recorded during maximal or submaximal exercise testing. In addition, the results suggest that multiple factors within each of the different physiological systems in the body are important in the fatigue processes during endurance activity. Within the brain, for example, a network of areas in multiple frequency bands is activated with fatigue.

Therefore, the most important finding of this thesis is that multiple different physiological and performance variables, associated with different physiological systems, are involved in endurance performance, and hence fatigue resistance. The involvement of multiple systems in endurance performance, and the many and varied physiological differences between the black and white South African runners, suggest that the processes and mechanisms involved in fatigue during endurance activity cannot be accounted for by any single factor, and that fatigue resistance is instead of a multifaceted and complex nature.

CONCLUSION

The findings of this thesis were that cardiorespiratory, intramuscular and neuromuscular factors were all associated with endurance performance, and hence fatigue resistance, and that there was activity in multiple brain regions common to subjects during fatiguing exercise. This is inconsistent with the theory that any single system can be the sole source of fatigue, and consistent with the premise that fatigue involves multiple mechanisms from different physiological systems. This research also found that multiple physiological or performance variables were significantly different between black and white South African runners, suggesting that the observed endurance performance difference between these ethnic groups may result from many, rather than a single, physiological factor. In addition, the factors that were different between the two ethnic groups, and the factors that were significantly related to endurance performance, were varied in nature, suggesting that integration of considerably differing factors is occurring during fatigue-associated processes during endurance activity. A model of the progression of the physiological changes associated with fatigue during endurance activity is proposed, and a role for the brain in integrating the physiological information from the body is suggested. The reason for the involvement of multiple physiological processes in fatigue is suggested to be the efficient maintenance of relative homeostasis in the body.

Chapter 1 Introduction

1.1 OVERVIEW

Fatigue is a universal element of human life. While known in the exercise sciences as a limiting factor for athletic performance, fatigue is a much farther-reaching phenomenon. It affects people from all cultures and from all socioeconomic backgrounds. It affects healthy people and those of ill health, being a symptom of many diseases, and is regularly reported in medical and psychiatric practices [143,190,259,289]. Exercise can play a role in the treatment or prevention of modern chronic diseases, many of which are common and costly to society [45,144,145,182], and it appears that one of the main reasons for the improved quality of life with exercise in patients is simply decreased fatigue [398].

Fatigue also affects people's capacity for work in many different careers. The loss in work productivity due to fatigue, combined with the financial pressure fatigue exerts on the health industry, can result in a significant economic burden at the level of the individual, the level of the community and even the level of the government [266]. In addition, although human-error accidents are generally in the realm of sleep-related fatigue [78,111], accidents with tragic or financial consequences may also result from activity-related fatigue, for example during the work of manual labourers. Fatigue is indeed a ubiquitous 'problem' for people the world over, with large-scale socioeconomic ramifications. Research into the causes of and possible counter-measures for fatigue will therefore prove worthwhile from a health, a sporting and an economic perspective.

Due to the multifaceted nature of fatigue, the study of this phenomenon is multidisciplinary [122]. However, perhaps the easiest way to study fatigue is to use the model of the endurance athlete, a healthy individual who regularly strives to overcome fatigue. Fatigue can be induced in this model by asking the athlete to exercise, and we can measure physiological aspects of fatigue by studying the exercising athlete under laboratory conditions. As already mentioned, fatigue with physical activity can affect anyone, from a manual labourer carrying boxes, to an elderly person climbing stairs, to an athlete competing in an Olympic event. However, the fatigue experienced by these

different individuals is qualitatively similar. It is therefore hoped that by investigating fatigue in athletes during endurance exercise we can shed light on the mechanisms of fatigue for the benefit of both sports people and the rest of the community.

There are many different types of tests that exercise scientists use to assess fatigue or differences in rates of development of fatigue. There are field-based and laboratory-based measures of performance. There are maximal and submaximal tests, and there are dynamic and static tests. Trials may use one, or a number, of the many different muscles of the body. Tests may consist of an open loop or a closed loop format. Similarly, a range of physiological variables can be measured during these tests in order to elucidate the metabolic workings of the body during fatigue. The tests used and variables measured in this thesis were chosen based on their relevance to the thesis and the availability of equipment in our laboratories. Technically, during these tests, physiological variables were measured rather than 'fatigue' itself, and endurance performance during exercise was measured rather than actual 'fatigue resistance'. This is due to fatigue itself not necessarily consisting of anything measurable. Indeed, the definition of fatigue is still controversial [411].

Fatigue has been defined in many different ways (Table 1.1). It has often been described as a form of acute physical failure or acute reduction in physical capacity [1,31,46,118]. In these definitions fatigue takes the form of a performance decrement. Fatigue has also, in contrast, been defined as "a symptom reported by subjects in whom there may be no obvious defect in muscle performance" [157]. This suggests that fatigue may be occurring whether or not a performance decrement is observed. Fatigue may consist of the various physiological processes occurring during endurance activity. Fatigue has also been suggested to be a sensation, rather than a physical process, with this sensation reflecting the conscious awareness of changes in subconscious homeostatic control mechanisms [412]. This sensation, however, may function as an active regulator of exercise intensity, rather than a passive consequence of metabolic processes during endurance activity, such that it ensures that exercise terminates before homeostatic failure occurs [411,412]. Therefore, while fatigue has generally been viewed as a negative entity, it could also be regarded as a positive phenomenon due to it playing a protective role in the body during the stress of exercise. In this thesis, fatigue has been analysed and discussed taking all of these definitions into account, although fatigue as 'an

amalgamation of physiological processes' and fatigue as 'a sensation' have dominated over fatigue as 'a physical failure'. Throughout the thesis, therefore, endurance performance has been used as a proxy for fatigue resistance, seeing as these two factors can be regarded as analogous, in practical terms. This explains the title of this thesis: "Endurance performance: the integrative physiology of resisting fatigue". By managing to successfully resist fatigue, and individual essentially demonstrates successful endurance performance.

Table 1.1: Definitions of fatigue during physical activity.

Definition of Fatigue	Reference
A form of acute physical failure or acute reduction in physical capacity	[1,31,46,118]
A symptom reported by subjects in whom there may be no obvious defect in muscle performance	[157]
A sensation reflecting the conscious awareness of changes in subconscious homeostatic control mechanisms	[412]
A sensation functioning as an active regulator of exercise intensity, rather than a passive consequence of metabolic processes during endurance activity, such that it ensures that exercise terminates before homeostatic failure occurs	[411,412]

There are a multitude of potential elements involved in endurance ability, from physiological factors to biomechanical, nutritional and psychological factors. In this thesis, a greater emphasis is placed on the physiological factors associated with superior endurance ability. A number of biomechanical factors have, however, been examined. In the subsequent text of this thesis therefore, biomechanical factors are included within the term 'physiological factors'. There are many candidate physiological variables that could play a role in fatigue, involving mechanisms related to substrate availability, metabolite accumulation, oxygen supply, muscle contractility and neural drive [65,122,141,255]. Multiple physiological systems work in concert to regulate the functioning of the body, and certain factors from some of these systems have been investigated in the different chapters of this thesis. The number of factors in each physiological system that could be involved in fatigue is vast, and investigating all of these factors is beyond the scope of this thesis. Consequently, this work has not comprehensively investigated each of these physiological systems, but rather highlighted aspects from each system that may play a role in fatigue in order to illustrate how varying factors may function in an integrated manner during fatigue. Potentially

relevant cardiorespiratory, intramuscular, neuromuscular and central nervous system factors have been examined. In the process, this thesis follows a similar path to the progression of exercise science in the twentieth, and now the twenty-first, century.

In the first half of the twentieth century the work of Nobel Laureates AV Hill, A Krogh and O Meyeroff contributed greatly to the understanding of changes in oxygen consumption during exercise [21,62,170,182,191,192,335]. The research of this thesis begins along a similar line in Chapter 2 by investigating cardiorespiratory factors involved in endurance performance. While this area has already been well studied in exercise physiology, re-exploring it establishes a base from which to build the subsequent chapters, and allows comparison with previous work. This also allows for the integration of cardioprespiratory factors with physiological factors from other systems in the body, as conducted in Chapter 6.

While the physiology of exercising skeletal muscle was studied by scientists, including those mentioned above, in the first half of the twentieth century, models of human muscle metabolism were generally deduced from measurements of the blood and expired air, or from animal muscle [62,182]. However, in the 1960's new experimental approaches using the percutaneous needle biopsy technique allowed for the study of many factors such as muscle protein contents, substrate levels and fibre composition using biochemical and histochemical techniques [24,25,125,182]. Chapter 3 of this thesis similarly investigated fibre composition and the content of certain proteins in skeletal muscle, making use of more recent developments and knowledge in muscle physiology. The term 'intramuscular' has been used in this chapter to distinguish the physiological factors examined in this chapter from those of the Neuromuscular factors chapter (Chapter 4). In the context of this thesis, therefore, 'intramuscular' refers to all parts of the muscle cell, including the sarcolemma.

Another Nobel Laureate, JC Eccles, in 1963, linked muscle and nerve physiology with research on fibre type innervation and repolarisation of the cell membrane [182]. However, the stimulatory connection between muscle and nerve was established long before the twentieth century, and electrology techniques were being used in the study of electrical activity in muscle in the eighteen hundreds by people such as Duchenne de Boulogne and EJ Marey [81]. Modern electrophysiology techniques have been applied to exercise

physiology and have contributed greatly to recent advances in the knowledge of mechanisms related to fatigue [122]. Neuromuscular features of fatigue have therefore been examined in Chapter 4 of this thesis, using electromyographic and muscle stimulation techniques, and with emphasis on the models of central and peripheral fatigue.

The role of the central nervous system is probably the least understood of the roles played by the different physiological systems of the body in fatigue. Recent technological advances in the neurosciences, however, have opened the way for the study of chemical and electrical central nervous activity, and their part in exercise and fatigue [121]. Therefore, using modern electroencephalographic techniques, Chapter 5 of this thesis investigated fatigue-related activity in the brain during exercise.

Chapters 2 to 5 have, like much of exercise physiology research, used a reductionist approach to investigating fatigue during physical activity, by examining individual variables or systems in the body. This physiological compartmentalisation, however, can make identification of the mechanisms of fatigue difficult [65]. Performing integrative studies of multiple physiological systems to investigate complex exercise-related mechanisms in humans can be technologically difficult, and therefore this type of research does not necessarily progress as rapidly as more reductionist approaches, that use, for example, cell culture techniques or isolated organs from animals [182]. However, it is likely that exercising metabolism is regulated in an integrated manner [255], and consequently research examining multiple physiological systems with an integrated approach is needed [182,413]. Therefore, Chapter 6 has investigated the concept that an athlete may resist fatigue well, and therefore perform well, as a result of the interaction of many variables from different physiological systems in the body. This chapter has examined the results from the previous chapters in an integrated fashion to address how the combined effect of multiple physiological factors can result in enhanced fatigue resistance and endurance performance ability.

1.2 THESIS FORMAT

This thesis consists of seven chapters. Chapters 2 to 5 investigate factors from different physiological systems in the body, namely the cardiorespiratory system, the intramuscular system, the neuromuscular system and the central nervous system, respectively. Chapter 6 addresses the integration of the different physiological systems, and Chapter 7 is the conclusion section of the thesis. Chapters 2 to 6 have a similar format to a journal paper, but with more detail. Due to the multifaceted nature of this thesis the literature review has been broken down into sections, and a relevant literature review included at the beginning of each of Chapters 2 to 6. For example, the relationship between cardiorespiratory variables and fatigue is reviewed at the beginning of the Cardiorespiratory factors chapter (Chapter 2), and the relationship between neuromuscular variables and fatigue is reviewed at the beginning of the Neuromuscular factors chapter (Chapter 4). In addition, Chapters 2 to 6 begin with a 'preamble', which introduces the chapter and links the findings of the previous chapter to the aim of the subsequent one.

1.3 SUBJECT SELECTION

Fatigue resistance has been described as a "crucial" element of distance running [338]. Indeed, long distance running is one of the most well known of the sports involving endurance ability, as well as one of the most studied sports in the exercise sciences. Distance runners were therefore used as subjects in this thesis for three reasons. Firstly, running is a fairly 'straightforward' movement that does not involve complicated skills or equipment (other than running shoes), simplifying measurements. Secondly, there has been much previous research on distance runners, allowing comparison of results with those from other studies. Thirdly, the location of our laboratory in South Africa allows access to a unique pool of distance runners, as will be described in more detail below. Male runners were selected, as this again allows for greater comparison with previous research than would be possible were female runners used.

In Chapters 2, 3 and 4 the potential relationships between physiological traits and endurance performance have been examined in two different ways. The results sections of these three chapters have therefore each been divided into two parts, A and B, to reflect these two methods. In part A the aim was to examine selected physiological variables and correlate them with field-based and lab-based measures of endurance performance. In order to do this, a subject group of 32 male runners, whose personal best times for a 10 km road race ranged from 30 to 41 min, was selected. Runners were chosen within this performance range as the testing protocol required them to exercise at various intensities, including high intensity. If the selection range had been broader (which would probably have increased the chance of achieving significant correlations between physiological factors and endurance performance), the slower runners might not have been able to complete the protocols.

Part B of Chapters 2, 3 and 4 were designed to take advantage of an opportunity available in this laboratory to study runners from a population group specifically known to perform well in long distance running. Over the second half of the twentieth century black athletes of African origin distinguished themselves increasingly in international running events of varying distances [338]. In South Africa specifically, black runners dominate the long-distance running events to an extent that cannot be dismissed as chance [47,84]. This disproportionate success of black South African runners compared to

runners from other ethnic backgrounds suggests that they may have some form of physiological framework that is advantageous to endurance performance, yet the nature of this advantage is not known. A few previous studies have examined physiological differences between black and white South African runners, concentrating mainly on cardiorespiratory and sometimes skeletal muscle differences [47,84,448,449], however the results have been inconclusive. Identifying which traits these black runners have that are associated with endurance performance and that are different to a 'control' group of white runners may suggest which physiological factors can be important in endurance performance in general, ethnicity aside.

Therefore, for part B of Chapters 2, 3 and 4, 16 black South African runners were recruited and matched for 10 km race time to a group of 16 white South African runners. It is acknowledged that the classification of the various ethnic groups or races is not absolute from a genetic or physiological perspective. The "black South African" runners studied in this thesis were all males living in the Western Cape of South Africa, of Southern African descent, with the majority being descended from the Xhosa tribe. The "White South African" runners were all Caucasian males living in the Western Cape of South Africa, of European descent (including English, Scottish, Irish, German and Dutch). The terms "black" and "white" will be used for the sake of brevity during the rest of this thesis. Bosch et al [47], Coetzer et al [84] and Weston et al [448] all compared black to white South African runners, but matched them differently. Coetzer et al [84] specifically wanted to examine elite black and white runners. As a result, they selected runners who were matched for their race times over distances of 1 to 5 km, but very different over longer distances. This was necessary because it would have been very difficult to match the fastest black runners over longer distances to white runners as there are not enough white South African runners who can perform at this same level, as will be highlighted below. Bosch et al [47] and Weston et al [448] instead chose to compare subelite black and white runners, matched for marathon and 10 km times, respectively. If the running performance of black and white distance runners in South Africa are both graphed, as shown in Figure 1.1, with number of runners on the y axis and 10 km race time on the x axis, a normal distribution would be evident for both. The distribution curve, however, would be shifted to the left for the black athletes compared to the white athletes, as on average the black runners outperform the white runners.

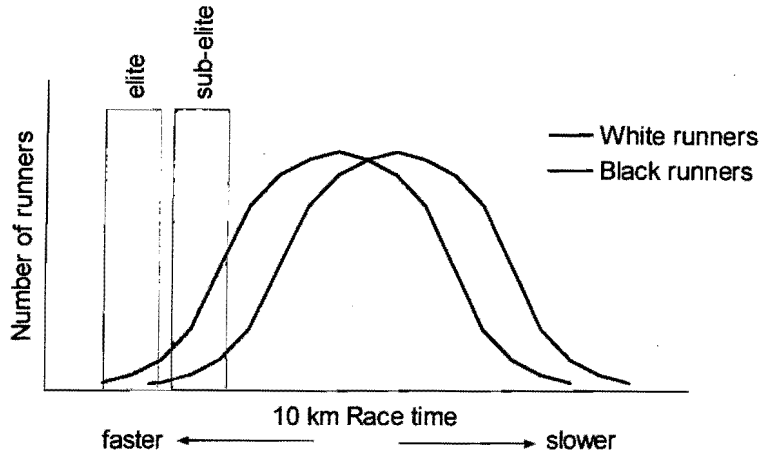


Figure 1.1: Distribution of black and white South African runners' 10 km times (hypothetical data).

It was decided that matching the black subjects to the white subjects for running time was necessary in this thesis, as a failure to do this would mean that potential physiological differences between the groups could be dismissed as merely being the result of the subjects being of different levels of running ability. It was therefore necessary to decide which level of running ability to match the two groups at. If recreational runners were selected from the middle of the performance range the chance of seeing any differences between the black and white groups would probably have been minimised. The two normal distribution curves exhibit a substantial overlap at this point, and differences between groups are probably more likely to be evident nearer the ends of the distribution. However, it would have been very difficult, if not impossible, to select runners from the very top of the range (the fastest runners) as this includes almost exclusively black athletes, as not enough white South African runners can perform at this same level. In addition, Bosch et al [47] suggested that any differences between groups should be apparent at the subelite, as well as the elite, level, as black runners also dominate over white runners at this level of performance. It has also been found that the physiological factors that determine success over distances of 10 to 90 km are the same [339], so runners from within this range should be selected.

Sub-elite runners, who could run 10 km in less than 41 minutes, were therefore selected, similar to Weston et al [448]. This allowed the use of relatively high performance runners while retaining access to enough subjects to include in the study, and any physiological differences found between the groups could be presumed to be due to an ethnic

difference and not a difference in running ability. In addition to investigating novel physiological variables not examined in the previous South African ethnic comparison studies, we have tested approximately double the subject numbers these studies used, so that their findings could be confirmed with a greater sample size.

There are two important factors to take into account during this ethnic comparison. Firstly, the definition of race is not absolute and the determination of an individual's ethnic group is never perfect. For example, defining an individual as 'black African' or 'white African' is not scientifically specific enough, as the athletic qualities of, say, west and east Africans are very different, with west Africans generally performing better in power rather than endurance events and east Africans vice versa [338]. The complete racial ancestry of the subjects in this thesis is not known and this limitation is acknowledged. The subjects were, however, all recruited from the Cape Town area and were all South African citizens, which will have minimised variations in ethnic background within the two groups, as well as the environmental differences between the groups.

Secondly, a confounding factor in the comparison of physiological differences between the ethnic groups in this thesis is the fact that the black subjects were significantly smaller (in mass and height) than the white subjects. This complication is challenging to avoid, as it is exceedingly difficult to match the two ethnic groups for mass and still retain viable subject numbers, due to the natural size difference that occurs between black and white South African males. Where a measured physiological variable in this thesis could be affected by body mass, either a correction for this has been made or it will be discussed in the results accordingly. It could also be argued that it is perhaps better not to match the ethnic groups for mass, as the chosen groups would then not be representative of their races, as a typical group of black runners would be smaller in size than a typical group of white runners.

The two groups of 16 runners from part B of Chapters 2, 3 and 4 together formed the group of 32 runners in part A. A different set of subjects from Chapters 2, 3 and 4 were tested in Chapter 5. The research for this chapter was conducted using new high-density electroencephalographic techniques in our laboratory in order to increase the knowledge of changes associated with fatigue in the central nervous system, specifically the brain.

Subjects of a wide range of athletic ability, from a broad age range, from both sexes, and of black, white and mixed racial ancestry, were tested. The protocol was designed such that healthy people of any level of athletic ability could complete it, and ethnic comparisons were not made. The aim was to determine how fatigue affects the brain in ways common to many different people.

1.4 OBJECTIVES

As described earlier in the introduction, many systems in the body have been investigated in an attempt to understand their role in fatigue. In many cases, studies have attempted to describe a single factor as the cause of fatigue [122]. Despite this, no agreement has been reached as to exactly what limits endurance performance or what causes fatigue during physical activity. This is probably because fatigue results from numerous different factors [65,122,141,255].

Therefore, the objectives of this thesis were to answer the following questions:

- Are multiple physiological factors associated with endurance performance?
- Are multiple physiological systems involved in endurance performance?
- Are the cardiorespiratory, intramuscular, neuromuscular and central nervous systems involved in endurance performance?
- Which of the measured factors from the cardiorespiratory, intramuscular and neuromuscular systems are associated with endurance performance?
- Which of the measured factors from the cardiorespiratory, intramuscular and neuromuscular systems are different between black and white South African distance runners?

And therefore:

- Is a single physiological factor likely to wholly account for fatigue during physical activity?
- Are the factors from a single physiological system likely to wholly account for fatigue during physical activity?

And for speculation (derived from the findings of this thesis and previous literature):

- What is the physiological reason for fatigue during physical activity?
- Why would multiple factors and systems be involved in fatigue during physical activity?
- How can the findings of this thesis be applied to athletes training to improve their fatigue resistance?

Chapter 2 Cardiorespiratory factors

2.1 PREAMBLE

The role of cardiorespiratory factors in endurance performance has probably been more extensively studied than the roles of factors from other physiological systems. In addition, most of the research that has attempted to uncover reasons for the superior endurance performance of athletes from certain ethnic backgrounds has concentrated mainly on cardiorespiratory variables [47,84,388,448]. Nonetheless, the research of this thesis has begun by briefly re-examining the part played by certain cardiorespiratory factors in fatigue during endurance activity. Re-exploring this area of study has allowed the formation of a base from which the subsequent chapters have been built, as well as allowing the establishment of the cardiorespiratory role in the integrated functioning of fatigue, in the Integration of systems Chapter (Chapter 6). The following literature review will address some of the cardiorespiratory factors related to endurance performance, concentrating on those variables measured in this chapter. As anthropometrical factors have also been examined in this chapter, the influence of anthropometry on endurance performance has also been briefly addressed at the end of this literature review.

2.2 LITERATURE REVIEW: The role of cardiorespiratory factors in fatigue and endurance performance

During exercise, an increase in oxygen and fuel consumption is necessary to meet the demands of an increased metabolic rate. Oxygen and fuels therefore need to be delivered to working muscles faster than when the body is at rest [65]. The oxygen delivery system consists of the respiratory and the cardiovascular systems. Oxygen is taken up by, and carbon dioxide removed from, the body by the lungs, while the circulation transports O_2 to the tissues and returns CO_2 to the lungs. The cardiovascular system also transports substances absorbed from the gastrointestinal tract to the tissues, removes products of metabolism to the kidneys, functions in the regulation of body temperature, and distributes hormones and other agents that regulate cell function [159]. During physical activity, the cardiovascular system increases blood flow to working skeletal and respiratory muscles, while blood flow is maintained or increased to the cardiac muscle and the brain [65]. Exercising muscles extract more O_2 from the blood, and an increase in ventilation provides additional O_2, eliminates heat, and excretes CO_2 [159]. The cardiovascular and respiratory systems must therefore operate and be regulated in an integrated manner during exercise in order to meet the oxygen and substrate needs of the active tissue, while removing CO_2, metabolic waste products and heat. There are multiple cardiorespiratory regulatory mechanisms to control these changes, and inadequate regulation of these mechanisms could result in symptoms of fatigue. On the other hand, efficient functioning of the cardiorespiratory system during exercise could, along with other factors, result in superior endurance performance due to successful resistance to fatigue [90].

The cardiorespiratory variables associated with endurance performance that have been investigated in this chapter are oxygen consumption (along with running economy), heart rate, respiratory exchange ratio. Due to their vascular location, plasma concentrations of lactate, sodium and potassium have also been examined in this chapter. The relationship between these factors and endurance performance will now be discussed.

2.2.1 Oxygen consumption

Oxygen consumption (VO_2) increases with increasing workload during exercise. Maximal oxygen consumption (VO_2max), or the maximum rate at which oxygen can be taken up and used by the body during exercise [65], is generally considered to be an index of cardiorespiratory, circulatory and muscular fitness [340]. Indeed, VO_2max has been shown to have a significant association with endurance performance, and can be improved with training [145,146,319,339]. The determinants of VO_2max are still debated, with the pump capacity of the heart, oxygen uptake by the lungs, blood oxygen transport and skeletal muscle factors all argued to play a role [107,108,389]. A good running economy, in other words a low VO_2 at any particular exercise intensity, may also be beneficial to endurance running [319,339]. It has been suggested that running success may, to a large part, be determined by VO_2max, running economy, and the ability to utilise a large fraction of VO_2max during competition [89].

The 'cardiovascular' or 'anaerobic' model of athletic performance proposes that the cardiovascular system has a limited capacity to supply oxygen to the active muscles, and therefore the capacity for prolonged work at submaximal intensities is strongly influenced by maximal oxygen uptake [23,336]. Noakes [337] has proposed an alternative physiological model in which skeletal muscle recruitment (and hence exercise performance) is regulated by a central "governor" specifically to prevent the development of myocardial ischaemia or skeletal muscle anaerobiosis during maximum exercise. While the relative importance of maximum oxygen consumption and other cardiorespiratory variables as limits to endurance performance is still being debated, the physiological importance of the cardiorespiratory system during physical activity is well established and accepted.

2.2.1.1 Oxygen consumption and ethnicity

Cardiovascular 'fitness' can be affected by ethnicity [134,362]. In a study of elite Kenyan and Scandinavian endurance runners, Saltin et al [388] reported no significant difference between the groups for VO_2max. However, running economy was different between the two groups, with the oxygen cost at a given running speed lower in the Kenyans than the Scandinavians, particularly at higher running speeds. Their study could not, however, explain the cause of the superior Kenyan running economy.

Several studies have compared oxygen consumption in elite and subelite black and white South African runners matched for race time [47,84,448,449]. The cardiorespiratory differences and other anthropometrical and physiological differences found between black and white South African runners in these studies are summarised in Table 2.1.

Table 2.1: Physiological and anthropometrical comparison of black and white South African runners: summary of the data from Bosch et al [47], Coetzer et al [84], Weston et al [448] and Weston et al [449]. "Black" refers to males living in the Western Cape of South Africa, of Southern African descent, and "white" refers to Caucasian males living in the Western Cape of South Africa, of European descent.

Measurement	Bosch (1990)	Coetzer (1993)	Weston (1999)	Weston (2000)
Body mass	black < white	black < white	black < white	black = white
Height	black < white	black < white	black < white	black < white
SSS	ND	black < white	ND	ND
% Body fat	black = white	ND	black = white	black = white
PTV	black < white	black = white	black = white	black = white
VO_2max	black = white	black = white	black = white	black < white
Fractional VO_2 utilisation	black > white	black > white	ND	black > white
Running economy	ND	black = white	ND	black > white
Fatigue resistance	ND	black > white	black > white	ND
Peak RER	black = white	black < white	black = white	black = white
Submax RER	black > white	black = white	black = white	black = white
Peak HR	black = white	ND	black = white	black > white
Submax HR	ND	ND	black = white	black ≥ white
Peak $_{pl}$[lactate]	ND	black < white	black = white	black = white
Submax $_{pl}$[lactate]	black < white	black ≤ white	black ≤ white	black = white
% Type 1 fibres	ND	black = white	black = white	ND
Isom quadriceps strength	ND	black < white	ND	ND

≤ and ≥ indicate that more than one similar measurement was made (e.g. at different exercise intensities) and only one/some were significantly different between groups. ND: not determined; SSS: sum of seven skinfolds; VO_2max: maximal oxygen consumption; PTV: peak treadmill velocity; RER: respiratory exchange ratio; HR: heart rate; $_{pl}$[lactate]: plasma lactate concentration. Fatigue resistance: time to fatigue during running (Weston, 1999) and isometric quadriceps contraction (Coetzer, 1993)

Bosch et al [47], Coetzer et al [84] and Weston et al [448] all reported no difference in VO_2max between black and white South African runners. While Coetzer et al [84] and Weston et al [448] also found no difference between the ethnic groups for peak treadmill running velocity, Bosch et al [47] reported a significantly lower peak treadmill running velocity in the black compared to the white runners. Bosch et al [47] also found that the black runners were able to run at a higher percentage of VO_2max (i.e. they had a higher fractional utilisation of VO_2) than the white runners during a treadmill marathon. Coetzer et al [84] similarly reported that the black runners had a higher fractional utilisation of VO_2 over distances greater than 5 km, but found no differences between the two groups of athletes for running economy. Weston et al [448] reported no difference between the ethnic groups for VO_2 at the same relative workload, but found that the time to fatigue during a submaximal running test was different between the groups, with the black runners lasting 21% of the total time longer. The authors suggested that this indicated a greater "fatigue resistance" in the black than the white athletes. The causes of the different fractional VO_2 utilisation and fatigue resistance in the black and white South African runners is not, however, clear.

In all these studies of South African runners, the black athletes had significantly lower body mass than the white athletes. Weston et al [449], however, investigated black and white South African runners who were matched for race time and for body mass (although not height), and reported a lower VO_2max in the black athletes than the white athletes. The black runners, however, were more economical than the white when running at 16.1 km/hr. Similar to the previous studies, the black runners utilised a higher percentage of VO_2 max than the white runners when running at their 10 km race pace.

Distance running performance is therefore affected by various factors related to oxygen consumption, including VO_2max, running economy and fractional utilisation of VO_2. The relative importance of these variables in endurance performance is still uncertain, but they appear to play a role in the variations observed in performance between different people as well as different ethnic groups.

2.2.2 Heart rate and respiratory exchange ratio

With the increase in oxygen consumption during exercise, a concomitant increase in oxygen delivery to the muscles is necessary. Ventilation and cardiac output therefore increase to meet the oxygen needs of the muscle. Heart rate (HR) is the major determinant of cardiac output during moderate to maximal exercise [65]. HR therefore increases with exercise intensity to enhance blood flow and therefore oxygen transport to the tissues where it is needed [65]. Peak HR tends to decrease with age, and HR is sometimes used as an estimate of the relative intensity at which an individual is exercising, although it can be influenced by factors other than simply exercise intensity [159].

External respiration refers to the absorption of O_2 and removal of CO_2 from the body as a whole, while internal respiration refers to the utilisation of O_2 and production of CO_2 by cells and the gaseous exchanges between cells and their fluid medium [159]. Indirect calorimetry is commonly used to measure oxidative fuel utilisation in humans by examining the ratio of CO_2 to O_2 in the expired gases, however this measure can be affected by acid-base disturbances so that it does not reflect only the cellular oxidation of specific fuels [317]. The term 'respiratory exchange ratio' (RER) therefore describes the ratio of the volume of CO_2 produced to the volume of O_2 consumed per unit of time, taking the measurement inaccuracies into account [293]. The RER provides a guide for estimating the nutrient mixture catabolised for energy during rest and exercise, particularly the relative quantities of carbohydrate and fat [293]. The RER increases with exercise intensity and generally ranges between 0.70 and 1.00, although during intense exercise it can exceed 1.00 [293].

2.2.2.1 Heart rate, respiratory exchange ratio and ethnicity

In a study of black and white South African runners matched for marathon time, Bosch et al [47] found that the black runners had a higher RER during submaximal exercise, suggesting a greater oxidation of carbohydrate relative to fat than the white runners. Coetzer et al [84] reported a significantly lower peak RER in black compared to white South African middle-to-long distance runners matched for race time. In contrast to both these studies, Weston et al [448] reported no significant difference between black and white South African runners matched for 10 km race time for peak RER or RER during

submaximal exercise intensities. It is not evident whether the differing results of these studies is the result of differences in subject selection, or instead due to the use of relatively small sample sizes. Weston et al [448] also reported no significant difference between the ethnic groups for peak HR or HR during submaximal exercise. When matched for body mass as well as race time, however, black South African runners were found to have a higher HR than white runners when running at their 10 km race pace, suggesting that the black runners were running at a higher relative intensity than the white runners [449]. Although they had a higher HR, the black athletes had a similar RER to the white athletes at this pace, suggesting that the two groups had the same carbohydrate flux, despite the black athletes running at a higher intensity.

Similar to oxygen consumption, HR and RER increase with exercise intensity. While oxygen consumption and HR are used to estimate relative exercise intensity, RER instead provides an estimate of the relative oxidation of fuels. Therefore, while HR and RER do not directly affect endurance performance or fatigue, they are measures used to assess physiological changes during endurance activity.

2.2.3 Plasma lactate concentration

One of the physiological changes that occur with physical activity is an increase in the skeletal muscle production of lactate. Lactate is produced in skeletal muscle during glycolysis and glycogenolysis. The lactate levels in the cytosol must be prevented from rising to too great an extent for high rates of glycolysis to be maintained, as is necessary during exercise. Lactate is transported across the sarcolemma into the blood, and is transported by the blood from tissues where it is produced to tissues where it is oxidised [62]. The increase in plasma lactate concentration that occurs during exercise is therefore a result of the rate of lactate appearance increasing faster than the rate of lactate disappearance [417]. The rise in plasma lactate with exercise will be affected by the type and intensity of exercise, as well as the environmental conditions the activity is performed in [286].

An increase in muscle lactate production during exercise is associated with an increase in H^+ ions, which could upset the pH of the cell [80], and contribute to fatigue during exercise [302]. As lactate and H^+ are cotransported across the sarcolemmal membrane into

the blood down proton and lactate concentration gradients [62], efficient channeling of lactate by the blood from tissues where it is produced to tissues where it is oxidised could help to delay fatigue. Lactate metabolism will be discussed in more detail in the Intramuscular factors chapter (Chapter 3), with specific reference to lactate transport by monocarboxylate transporters. Plasma or whole blood lactate concentration is measured more often in exercise physiology research than muscle lactate concentration, because it is a simpler and less invasive measure. Although blood lactate concentration cannot indicate muscle lactate concentration, it does give an indication of lactate flux in the body.

2.2.3.1 Plasma lactate concentration and ethnicity

There are ethnic differences in the increase in blood or plasma lactate concentration during exercise [47,84,193,388,448]. Saltin et al [388] found that blood lactate concentrations were lower in elite Kenyan than Scandinavian runners at a given submaximal exercise intensity. However, no differences were found between groups for peak blood lactate values. Differences in exercising blood lactate concentrations have also been reported between black and white South African runners.

Bosch et al [47] reported lower blood lactate levels in black than white subelite South African marathon runners after a treadmill marathon. Similarly, Coetzer et al [84] found lower blood lactate concentrations in black compared to white elite South African runners after running at 21 km/hr and after a maximal running test. The authors concluded that while the lower lactate levels in the black runners could be the result of a difference in the rate of lactate accumulation, it could also be the result of a difference in the rate of lactate removal from the blood or, indeed, the rate of lactate transport from the muscle into the blood. Lactate transport across the sarcolemma is facilitated by monocarboxylate transporter proteins [63], suggesting that variations in the activity or expression of these proteins in the muscle could be associated with the ethnic differences in plasma lactate concentration during exercise. This possibility will be discussed further in the Intramuscular factors chapter (Chapter 3).

In a study of subelite black and white South African runners, Weston et al [448] found that the black athletes had significantly lower plasma lactate levels than the white athletes after running at a high submaximal intensity. The black runners accumulated lactate at a

significantly slower rate than the white runners with increasing exercise intensity. An increase in skeletal muscle oxidative enzyme capacity may result in reduced lactate production [173]. In support of this, Weston et al [448] found citrate synthase activity to be 50% higher in the black runners compared to the white after running at the same high submaximal intensity. Despite these previous reports of ethnic differences in plasma lactate levels during exercise however, when comparing black and white South African runners matched for body mass, Weston et al [449] found similar plasma lactate concentrations between the black and white runners, when running at a 10 km race pace.

Therefore, lactate production in the skeletal muscle is increased during exercise, resulting in an increase in lactate transport into the blood, where the lactate is redistributed for oxidation. There are ethnic differences in the increase in plasma lactate concentration during exercise. As the concentration of lactate in the muscle and blood have been implicated in fatigue processes, it is possible that the lower blood lactate levels in black African distance runners could be related to their superior endurance performance compared to runners of other ethnicities.

2.2.4 Plasma sodium and potassium concentration

Similar to plasma lactate, the plasma concentrations of sodium and potassium may also change during exercise. These changes result from an imbalance between the intake and loss of sodium (Na^+), potassium (K^+) and water due to renal and/or extra-renal mechanisms [281]. Extra-renal mechanisms involve sweating, fluid ingestion and the movement of electrolytes between fluid compartments. In a study of men cycling for 45 min, Mallie et al [281] found that renal Na^+ and K^+ clearances decreased, but plasma sodium levels did not change. Based on these observations, they concluded that extra-renal mechanisms were probably the main causes of exercise-induced changes in plasma sodium concentration.

As a result of the different factors that can affect the influx and efflux of Na^+ to or from the blood, the change in plasma Na^+ levels during exercise varies depending on the individual performing the exercise, the type of exercise, the environment the exercise is performed in and the type and rate of fluid consumption [87,318,342,410]. For example,

Convertino et al [87] reported a curvilinear increase in plasma Na^+ concentration during graded cycle ergometer exercise, with a work intensity of 40 % of VO2max being enough to cause a significant increase. On the other hand, Speedy et al [410] found no significant difference between pre- and post-race serum Na^+ concentration in athletes competing in an Ironman triathlon.

Muscle contraction also affects plasma Na^+ and K^+ levels more directly via the generation and conduction of action potentials. Muscle action potentials result in an influx of Na^+ into, and an efflux of K^+ from, muscle cells [263]. During exercise, the release of K^+ ions from contracting muscle results in a decrease in intracellular K^+ concentrations and an increase in plasma K^+ concentrations [263,306,316]. The outward flux across the sarcolemma is mainly via the voltage-dependent K^+ channels associated with action potentials [213]. K^+ released to the blood accumulates in the plasma, although some may also be taken up by red blood cells [213].

Na^+ is transported back out of the muscle cell, and K^+ back into the cell, by Na^+/K^+ exchange pumps [65]. They are driven by ATP hydrolysis [65], and their activation works to restore the plasma and intracellular Na^+ and K^+ concentrations to pre-exercise levels. Inhibition of the Na^+/K^+ pumps or a reduction in the muscle concentration of functional Na^+/K^+ pumps decrease muscle excitability and the maintenance of force during continued stimulation [82]. Medbo et al [306] reported that peak post-exercise plasma K^+ concentration was linearly related to the exercise intensity. The authors suggested that the increased plasma K^+ concentration during exercise can be explained solely by the electrical activity in the exercising muscles and that rate of K^+ reuptake is proportional to the extracellular accumulation. Miyamura et al [316] found that blood potassium concentration was highest at one minute after exhaustive cycle exercise and returned to resting levels within a few minutes during recovery. A post-exercise (± 3 min after exercise) plasma K^+ concentration undershoot (compared to resting levels) sometimes occurs, and can be explained by a higher gain of the Na^+/K^+ pump after exercise [306].

Skeletal muscles contain the largest single pool of K^+ in the body and therefore play an important role in whole body K^+ homeostasis [213]. Lindinger et al [263] describe how increased interstitial K^+ concentrations in contracting skeletal muscle stimulate Group III and IV afferent nerves, which in turn stimulate heart rate and ventilation rate, resulting in

increased cardiac output and respiration. Along with the direct vasodilatory effect of K^+ on the vascular bed in the contracting muscles, this results in increased delivery of metabolic substrates to, and removal of metabolic endproducts from, exercising muscle tissue. In support of this, Miyamura et al [316] found that peak blood K^+ concentration correlated significantly with maximum pulmonary ventilation, while Juel et al [219] suggested that local blood flow could be regulated by the accumulation of K^+ in that region. While these effects can be beneficial in exercise, loss of K^+ from muscle cells can also be a factor that contributes to symptoms of muscle fatigue, by decreasing muscle excitability [301].

2.2.4.1 The effect of plasma sodium and potassium concentrations on fatigue

Changes in plasma K^+ concentrations during exercise may interfere with muscle membrane excitability and therefore contractile function [213]. The increase in extracellular potassium concentration with exercise should, in theory, cause a decline in the resting membrane potential of the sarcolemma [306,407]. This membrane depolarisation should lead to a decreased membrane excitability and impair propagation of the action potential, resulting in a decrease in force output from the muscle. This theory would therefore imply that increased extracellular potassium is a factor leading to muscle fatigue [263,300,408]. West et al [446], however, found that membrane excitability was not decreased with increased extracellular potassium concentration following a fatiguing isometric quadriceps contraction, which they presume was due to enhanced Na^+/K^+ATPase activity. They suggested that changes in potassium concentration might still be related to the development of fatigue, but by exerting an effect distal to membrane action potential propagation, possibly in the T-tubular region.

As mentioned earlier, changes in K^+ concentrations in contracting skeletal muscle stimulate Group III and IV afferent nerves. Via afferent connections to the central nervous system therefore, the loss of K^+ and gain of Na^+ by exercising muscle may add to the sensation of pain that can occur with prolonged exercise [263]. Lindinger et al [263] postulate that this effect is a fatigue 'safety mechanism', which decreases voluntary exercise intensity, thereby protecting the muscle from the potentially negative effects of continuing to contract in the face of metabolic insufficiency. Changes in the concentration of Na^+ and K^+ may also have a metabolic, rather than a neuromuscular, effect on fatigue processes. The intracellular decrease in potassium concentration and

increase in sodium and lactate concentration that occur during muscle contraction contribute to a rise in the intracellular hydrogen ion concentration [300]. This causes an intracellular acidosis, which has been linked to fatigue through impairment of regulatory and contractile protein function and calcium regulation [300].

2.2.4.2 The effect of training on plasma sodium and potassium concentrations

Training enhances K^+ regulation in muscle and blood, and this may result in improved endurance performance by reducing muscle fatigue [302]. Both short-term training [176] and prolonged endurance training [174] increased the Na^+/K^+ pump concentration in skeletal muscle. Seven weeks of cycle-sprint training was reported to cause an increase in muscle Na^+/K^+ pump concentration as well as a reduction in the exercise-induced increase in plasma K^+ concentration, consistent with improved plasma and skeletal muscle K^+ regulation [304]. The increase in Na^+/K^+ pump density with training has not, however, been proven to account directly for the reduced increase in plasma K^+ concentration during exercise [301]. Instead, an increased activation of the Na^+/K^+ pumps with exercise may be more likely to account for the improved potassium regulation after training [301]. Training has also been shown to affect exercising plasma sodium concentrations, with seven weeks of sprint training resulting in a reduced net loss of Na^+ from the plasma with exercise [303].

In addition to training, the muscle membrane Na^+/K^+ pump content is also increased by an acute bout of high intensity exercise, and it has been suggested that this is partly mediated by translocation of α- and β-subunits of the pump to the sarcolemmal membrane [217]. In contrast to trained endurance athletes, patients with McArdle disease have been found to have reduced skeletal muscle concentrations of the Na^+/K^+ pump compared to healthy individuals [180]. These patients were also found to have higher peak increases in plasma potassium concentration in response to cycle exercise, and these factors may play a role in the exertional fatigue experience by McArdle sufferers [180].

Plasma concentrations of sodium and potassium may therefore change during exercise due to a number of factors. These changes many be associated with the development of fatigue through a number of mechanisms, involving direct effects at the level of the muscle as well as indirect effects via afferent stimulation. Training enhances the regulation of Na^+ and K^+ in the muscle and blood, and this may result in improved

endurance performance by reducing muscle fatigue. The effect of ethnicity on exercising plasma sodium and potassium concentrations has not previously been compared in black and white South African runners, although it has been suggested that plasma potassium accumulation be studied in these groups [448].

2.2.5 Anthropometry

Different anthropometrical phenotypes are advantageous for different types of sports. The anthropometrical phenotype considered to be advantageous for performance in endurance running is one with low body mass, small stature and low body fat, as well as low endomorphy and high ectomorphy [16,338]. One of the main advantages of a small body mass in endurance running may be that lighter runners have a lower metabolic heat production [102], as the rise body temperature may be a limiting factor during exercise [226,283]. Indeed, Marino et al [285] found that the advantages of a small body mass are mainly important when endurance exercise is performed in a high temperature environment. In their study, heat storage was positively correlated with body mass at 35 °C, only moderately correlated at 25 °C, and no correlation was evident at 15 °C. They concluded that smaller runners have a thermal advantage compared to heavier runners, when running in conditions in which heat-dissipation mechanisms are being maximally utilised.

2.2.5.1 Anthropometry and ethnicity

Anthropometrical differences occur between ethnic groups [195,334,397]. Comparisons of black and white American athletes found a lower sum of skinfolds in the black athletes and a difference in fat patterning between the groups [195,334]. The black athletes also had a lower or equivalent body mass index and body fat percentage compared to the whites [195,334]. Schutte et al [397] reported that black students had a greater density of lean body mass than white students as a result of a greater bone density.

The anthropometrical phenotype of individuals of any ethnicity, however, depends on their ancestral geographical origin. The ancestry of the black North Americans described above, for example, is likely to stem from West Africa. In general West African athletes perform well in power sports and have a different anthropometrical phenotype to black East Africans or South Africans, who generally perform well in endurance sports [338].

Comparisons of anthropometrical differences between ethnic groups competing in the same sporting discipline, however, may reveal why some ethnic populations exhibit superior athletic performance over others.

Several studies have examined anthropometrical differences between black and white South African runners. Bosch et al [47] studied black and white subelite South African runners matched for marathon time and found that the black runners had significantly lower height and mass than the white runners. They also noted a difference in fat patterning between the two groups, with the black athletes having a lower skinfold thickness for thigh, calf and triceps. Coetzer et al [84] studied elite South African runners matched for their race times over distances of 1 to 5 km, and found that the black runners were significantly shorter and lighter, with a lower lean thigh volume and a lower sum of seven skinfolds, than the white runners. Weston et al [448] investigated subelite South African runners matched for 10 km race time. Similarly to Bosch's [47] and Coetzer's [84] results, they found that the black runners were significantly shorter and lighter than the white runners, but had similar percentage body fat. Durandt [114] investigated sedentary black and white South Africans and found, similarly to the differences reported for South African runners, that the black subjects were significantly shorter and lighter than the white subjects.

2.2.6 Summary

Exercise is associated with extensive adjustments in the cardiorespiratory system that function to meet the oxygen and substrate needs of the active tissue, and to remove CO_2 and heat from the body during exercise. Inefficient cardiorespiratory regulation during physical activity could result in symptoms of fatigue, while efficient regulation could aid fatigue resistance and hence endurance performance. Oxygen consumption, heart rate and respiratory exchange ratio are involved in the cardiorespiratory response to exercise, and will therefore play a role in fatigue, along with the flux of metabolites such as lactate, sodium and potassium in the blood. Physiological differences have also been found for these factors between different ethnic groups with different athletic performance abilities. Cardiorespiratory variables, specifically those described above, are therefore relevant to the study of endurance performance.

2.3 INTRODUCTION

Cardiorespiratory research in the exercise sciences has grown exponentially since the early experiments of A.V. Hill and colleagues in the 1920's. As a result of this research, various cardiorespiratory factors have been linked to endurance performance or to the fatigue that occurs during endurance activity, as described in the literature review section of this chapter.

In addition to the research linking cardiorespiratory variables to endurance performance, reports of ethnic differences in cardiorespiratory factors, including fatigue resistance and economy during running [388,448], suggest that there could be cardiorespiratory origins to the observed differences in distance running performance between ethnic groups [338]. The recurrent finding of low blood lactate levels with exercise in black African runners [47,84,388,448] also warrants further investigation.

This chapter therefore examined the relationships between physiological variables of the cardiorespiratory system and distance running performance. Variables measured in previous studies were repeated, partly because they were sometimes contradictory in these studies, and partly because it was important to have these measurements for the subject groups in order to relate them to the data from the other physiological systems in the subsequent chapters. In addition, the exercising plasma concentrations of sodium and potassium, which have not previously been compared in black and white South African runners, were investigated.

This chapter therefore examined oxygen consumption (along with running economy), heart rate, respiratory exchange ratio, plasma lactate concentration, plasma sodium concentration and plasma potassium concentration. These physiological variables were correlated with subjects' 10 km personal best running times as well as with their peak treadmill velocities to relate them to both a field-based and a laboratory-based performance measure. Noakes et al [339] have found that peak treadmill velocity is the best laboratory-based predictor of performance in 10 km races. Furthermore, differences between these cardiorespiratory variables in black and white South African runners were examined to determine if they could be linked to the superior performance of black South African runners.

2.4 METHODS

2.4.1 Subject characteristics

Thirty-two (sixteen black and sixteen white) South African long distance runners were recruited from the Western Cape running community through advertising and communication with local coaches. All subjects were male, between the ages of 16 and 40 and had run a 10 km road race in less than 41 minutes within the previous two years. A translator was present when testing subjects for whom English was not a first language. The study was approved by the Ethics and Research Committee of the University of Cape Town Faculty of Health Sciences, and all subjects signed an informed consent form translated into their home language (Appendix A, Form 1 and Form 2) prior to participation in the study. All 32 subjects were initially analysed together as a single group (Part A), and subsequently analysed as a group of 16 black runners and a group of 16 white controls (Part B). The reason for dividing the groups in this manner is described in the subject selection section of the thesis introduction (section 1.3).

2.4.2 Experimental design

All subjects visited the laboratory on three occasions, with at least two days between the second and third visits (Figure 2.1). Subjects were instructed not to exercise in excess of their normal activity in the two days prior to a testing session. During the first visit subjects signed informed consent (Appendix A, Form 1 and Form 2) and filled in a questionnaire regarding their personal details, medical and injury details, and race times (Appendix B). Anthropometry measurements were performed on the subjects (section 2.4.3) and they were subsequently familiarised with running on the treadmill. On the second visit subjects performed a maximal running test on a treadmill (section 2.4.4.2), during which oxygen consumption (VO_2), VCO_2 (for calculation of respiratory exchange ratio (RER)) and heart rate (HR) were measured and blood samples were taken for analysis of plasma concentrations of lactate, sodium and potassium. On the third visit subjects reported to the laboratory in the morning, after having fasted for 10 hours to minimise the influence of recent food consumption on RER measurements, and had their resting VO_2 and VCO_2 (RER) measured (section 2.4.4.1). They then performed a submaximal run on the treadmill (section 2.4.4.3) during which VO_2 and VCO_2 (RER) were measured, and an interval run (section 2.4.4.4) during which VO_2, VCO_2 (RER) and HR were measured and blood samples were taken for analysis of plasma concentrations

of lactate, sodium and potassium. Three subjects were unable to return for the third day of testing and were therefore not included in the analysis of data collected on the third testing day.

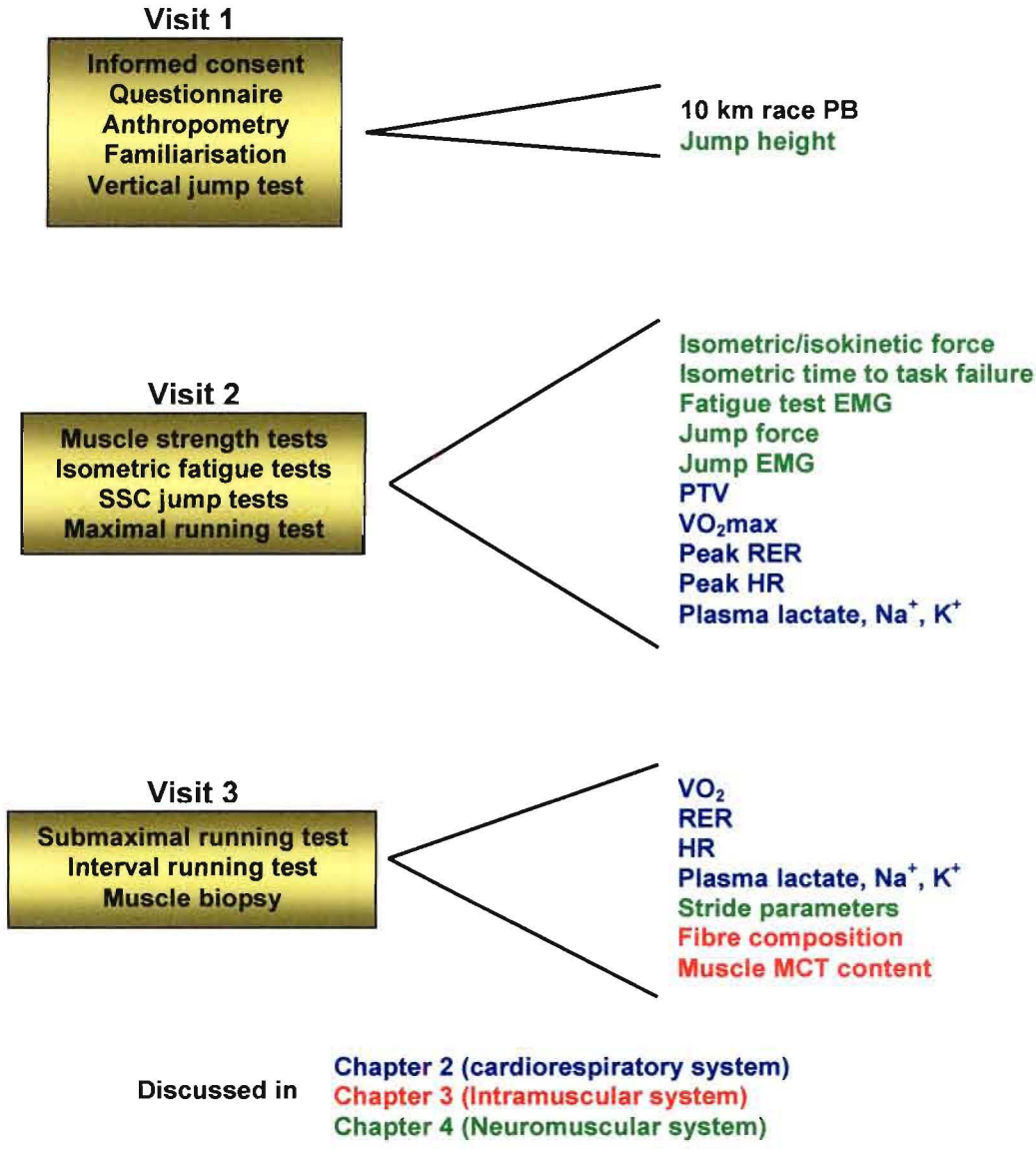


Figure 2.1: Experimental design for the Cardiorespiratory, Intramuscular and Neuromuscular Factors Chapters (Chapters 2, 3 and 4), including the tests conducted on the subjects during their three visits and the measurements performed during those tests. SSC: Stretch-shortening cycle; PB: Personal best time; EMG: Electromyography; PTV: Peak treadmill velocity; VO_2: Oxygen consumption; RER: Respiratory exchange ratio; HR: Heart rate; MCT: Monocarboxylate transporter

2.4.3 Anthropometry

The subjects' height and weight were measured. Skinfold thickness measurements were performed on each subjects at the biceps, triceps, subscapular, supra-iliac, abdominal, mid-thigh and calf sites [375]. Body fat was expressed as the sum of seven skinfolds (SSS) and as a percentage based on the method of Durnin and Womersley [115]. While it has been suggested that ethnicity-specific equations are needed when calculating percentage body fat in populations of different ethnic backgrounds [334], there are, to our knowledge, no such ethnicity-specific equations or correction factors to apply differently to the two populations we have studied. We have therefore included the sum of skinfolds measurement with the percentage body fat measurement so as not to be misled by any possible fat estimation errors that could occur with the application of body fat equations to the different ethnic groups. Forearm, sub-gluteal, mid-thigh, above-knee and calf girths along with the sub-gluteal to above-knee height were used for the calculation of muscle mass [288]. Contracted upper-arm girth along with femur, radius/ulnar, transverse chest and anterior/posterior chest diameters were measured for calculation of somatotype [186]. This included the calculation of scores for ectomorphy (the relative linearity of an individual's physique), mesomorphy (the relative muscularity of physique) and endomorphy (the relative 'roundness' of physique). Lean thigh volume (LTV) was calculated using the assumption that the thigh was the shape of a truncated cone, based on the method adapted from Katch and Katch [225] by Coetzer et al [84] and validated against LTV assessed by magnetic resonance imaging [237]. All measurements were performed by the same investigator to remove inter-observer error. Calculations were performed using specifically developed software [368].

2.4.4 Resting and running measurements

As described previously, VO_2 (ml/kg/min) and VCO_2 (RER) were recorded during four separate tests: 1.) a resting measurement, 2.) a maximal running test, 3.) a submaximal running test and 4.) an interval running test. The VO_2 and VCO_2 (RER) readings were recorded at ten second intervals throughout all four tests using a gas analyser (Oxycon Alpha automated gas analyzer, Oxycon, Jaeger, The Netherlands). The gas analyser was calibrated before each testing session using a Hans Rudolph 5530 3-litre syringe and a two point calibration technique, using 5% CO_2 – 95% N_2 gas mixture and fresh air. The subjects wore either a mask that covered their mouth and nose or a mouthpiece with a nose clip, which was connected via twin tubing to the gas analyzer and online

computer. The analyser software corrected the data for the appropriate dead space volume. VO_2 and RER were calculated using conventional equations [443]. As the black and white subject groups in part B had a different mean body mass, VO_2 was expressed in ml/kg/min and not l/min. HR data (beats per minute) was collected during the maximal running test and the interval running test at five second intervals using a Polar Sport Tester heart rate monitor (Polar Electro, OY, Kempele, Finland). Data was downloaded onto a personal computer and analysed using Polar Precision Performance software (Polar Electro, OY, Kempele, Finland).

2.4.4.1 Resting measurement:

Resting VO_2 and VCO_2 (RER) were measured to identify baseline levels of oxygen and fuel utilization in all subjects. The subjects presented at the laboratory in the morning after fasting for ten hours and readings were taken over a period of ten minutes with the subjects seated and breathing normally. The resting VO_2 and RER data from the last three minutes of the rest period (when the values had reached steady state) were averaged to obtain mean resting values.

2.4.4.2 Maximal running test:

Subjects were allowed time to stretch and warm-up according to their preferred routines, which included a period of jogging on a motorized treadmill (Quinton Instruments, Seattle, U.S.A) before beginning the maximal running test. The test used was the protocol described by Noakes et al [339], with a modification of the starting speed. Subjects began running on the treadmill at a 0° gradient at 10 km/hr (instead of 12 km/hr as used by Noakes et al [339]). The treadmill velocity was increased from the starting velocity by 0.5 km/hr every 30 seconds until the subject could no longer maintain the necessary speed and stopped running. VO_2, VCO_2 (RER) and HR were measured throughout the test. This data was used to determine the peak VO_2, RER and HR reached by the subjects during the test, which were recorded along with their peak treadmill velocity (PTV). Peak VO_2 and RER were measured to identify maximal levels of oxygen and fuel utilisation in the subjects. The relationship between these variables, as well as maximal HR, and running performance measures such as PTV could then be examined. Blood samples (10 ml) were taken before, immediately after and three minutes after the maximal run and analysed for plasma concentrations of lactate, sodium and potassium as described in section 2.4.5.

2.4.4.3 Submaximal running test:

Subjects were allowed time to stretch and warm-up according to their preferred routines, which included a period of jogging on the treadmill before beginning the submaximal running test. The test consisted of a 15 minute run on the treadmill at a 0° gradient at 50% of their peak treadmill velocity. The VO_2 and RER data from the last three minutes of the run (when the values had reached steady state) were averaged to obtain mean values. As this data was recorded at 50% of each subject's PTV, it allowed comparison of the runners' oxygen consumption and fuel utilisation at the same relative submaximal exercise intensity.

2.4.4.4 Interval running test:

Subjects were allowed to rest for a few minutes after completing the submaximal running test before performing the interval run. For the interval run, subjects ran at 10, 12, 14 and 16 km/hr, for five minutes at each speed with a five minute rest in between each run (in order of increasing speed). VO_2, VCO_2 (RER) and HR were measured during each run stage. The VO_2 and RER data from the last minute of each speed interval (when the values had reached steady state) were averaged to obtain mean values. These cardiorespiratory variables (VO_2, RER and HR) have been expressed in two different ways in the results. They are first expressed as absolute values. The subjects all ran during this test at the same absolute velocities, which allowed comparison of the runners' oxygen consumption, fuel utilisation and heart rate at the same absolute submaximal exercise intensities. This allowed comparison of running economy between the ethnic groups at four different speeds. When calculating running economy, it may appropriate to scale oxygen consumption per $kg^{0.66}$ rather than per kg due to the relationship between metabolic power and body mass [449], so VO_2 at each running speed has been expressed both per kg and per $kg^{0.66}$. Secondly, for each set running speed during the interval run, the percentage of each subject's PTV that the speed represented was calculated (%PTV). The subjects' cardiorespiratory variables (VO_2, HR or RER) were then divided by this %PTV value and graphed as this ratio. This allowed a relative representation of the subjects' cardiorespiratory variables, as it corrected for the fact that 12 km/hr, for example, may have been 50% of one subject's PTV and 45% of another's PTV. Blood samples (10 ml) were taken before the interval run, immediately after each of the interval run stages and four minutes after each of the run stages and analysed for

plasma concentrations of lactate, sodium and potassium as described in section 2.4.5. These blood metabolite variables have been expressed in the same two ways as the cardiorespiratory variables in the results, namely as absolute values and per %PTV.

2.4.5 Blood sampling and analysis

Prior to the maximal running test and the interval running test a cannula was inserted into each subject's antecubital vein. Blood samples were collected (at the time points described in sections 2.4.4.2 and 2.4.4.4) for the measurement of venous plasma lactate, sodium and potassium concentrations. For each 10 ml sample, 5 ml was transferred into a potassium oxalate and sodium fluoride vacutainer tube for plasma lactate concentration determination and 5 ml into a lithium heparin vacutainer tube for plasma sodium and potassium concentration determination. Tubes were stored on ice immediately after collection and centrifuged at 3000 rpm for 10 minutes at 4°C, after which the plasma fraction was pipetted into eppendorff tubes for storage at -20°C until analysis. Plasma lactate concentrations were determined via enzymatic spectrophotometric measurements using a commercial kit (Lactate Pap, Bio Merieux, Marcy-L Etiole, France). Plasma sodium and potassium concentrations were analysed using an EasyLyte PLUS Na/K/Cl analyzer (Medica corporation, Bedford, MA, USA).

2.4.6 Statistical analysis

Statistical analyses were performed using the Statistica software package (Version 6, Statsoft, Tulsa, OK, USA). Correlations between physiological variables and performance variables (independent and dependent variables) in both part A and B were performed with the Pearson Product Moment Correlation. When the data was divided into two groups based on ethnic origin (part B), comparisons of variables between the two groups were performed using the unpaired Students' t-test. Statistical significance was accepted when $p<0.05$. Four of the subjects did not complete the maximal running test due to difficulty with running at high speed on the treadmill and their data for this test was excluded from all subsequent analysis. Blood samples were not collected for all subjects at all time points due to cannula blockage and vasoconstriction. This missing data was therefore also not included in statistical analysis, as reflected in the subject number (n) values in the results section of this chapter. In addition, haemolysis of some of the plasma samples for sodium and potassium analysis occurred and this data was also excluded from analysis.

2.5 RESULTS

2.5.1 Part A: Physiological variables and endurance performance

2.5.1.1 Anthropometry

Subjects' anthropometrical variables are shown in Table 2.2.

Table 2.2: Anthropometrical variables of all subjects (n=32). Values expressed as mean ± standard deviation.

Anthropometric variable	
Age (yrs)	22.9 ± 5.4
Height (cm)	174 ± 9
Weight (kg)	64.9 ± 10.5
BMI (kg/m^2)	21.4 ± 2.1
LBM (kg)	56.9 ± 8.8
% Body fat	12.2 ± 3.1
SSS (mm)	51.0 ± 14.6
% Body muscle	54.7 ± 5.0
LTV (cc)	3566 ± 572
LTV/LBM (cc/kg)	63.3 ± 9.6
Endomorphy	2.32 ± 0.87
Mesomorphy	4.35 ± 1.24
Ectomorphy	3.24 ± 0.95

BMI: body mass index; LBM: lean body mass; SSS: sum of seven skinfolds; LTV: lean thigh volume

The mean 10 km personal best time (PB) of all the subjects was 2078 ± 193 s (34 min 38 s), with a range of 1815 – 2455 s (30 min 15 s – 40 min 55 s). The subjects' anthropometrical variables that are related to body fat content, namely percentage body fat ($p<0.001$), sum of seven skinfolds (SSS, $p<0.001$) and endomorphy ($p<0.01$) were positively correlated with their PB (Figure 2.2 a, b and c), while those related to body musculature, namely lean thigh volume (LTV, $p<0.05$) and the lean thigh volume to lean body mass ratio (LTV/LBM, $p<0.01$), were negatively related to PB (Figure 2.2 d and e). None of the other measured anthropometrical variables correlated significantly with the subjects' PB (data not shown).

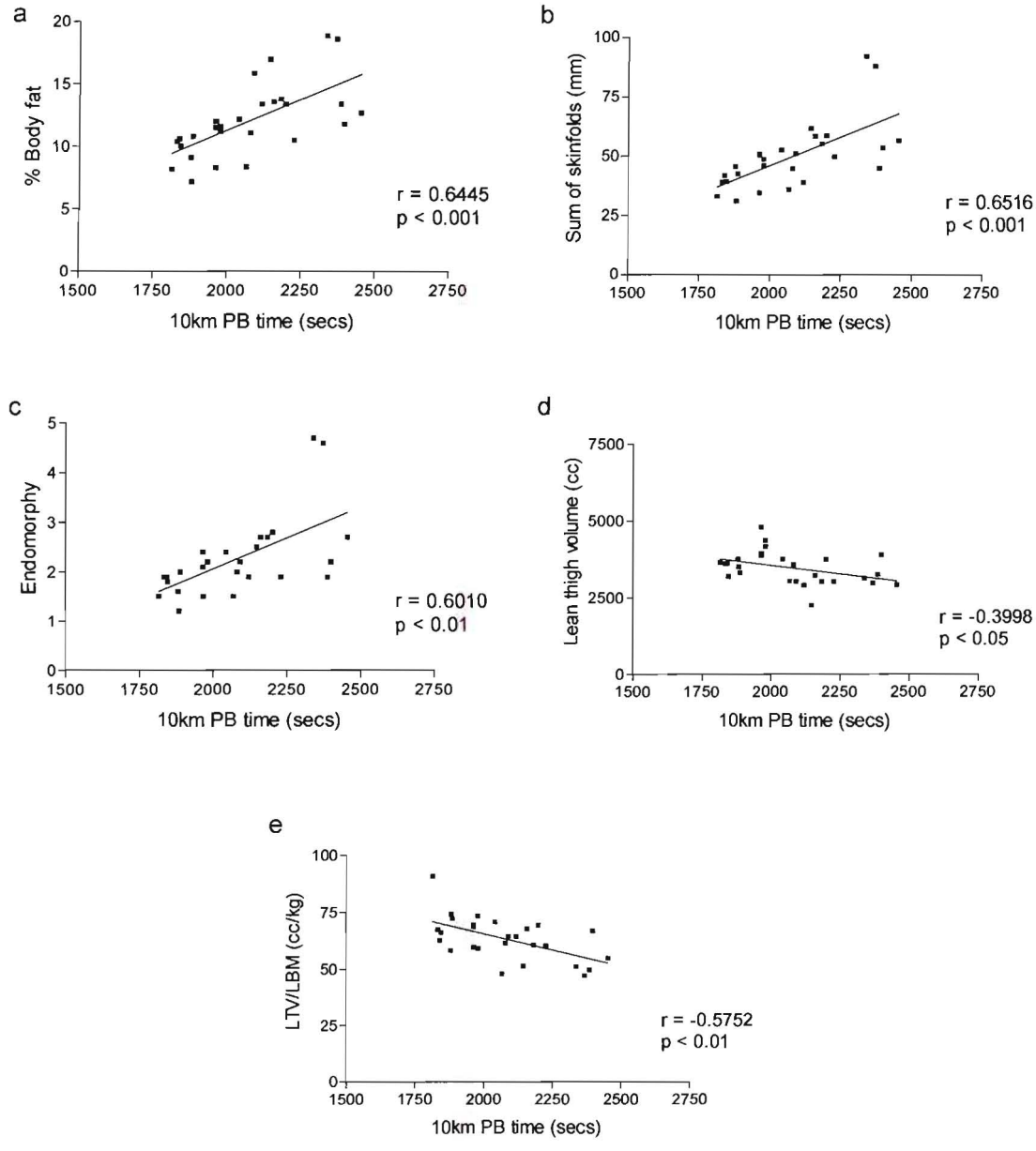


Figure 2.2: Correlation of 10 km personal best time (PB) with anthropometrical variables (n=27), namely: percentage body fat (a), sum of seven skinfolds (b), endomorphy (c), lean thigh volume (d) and lean thigh volume/lean body mass (LTV/LBM, e).

The only anthropometrical variables that correlated significantly with the lab-based measure of running performance, PTV, were percentage body fat ($p<0.05$) and mesomorphy ($p<0.05$) (Figure 2.3).

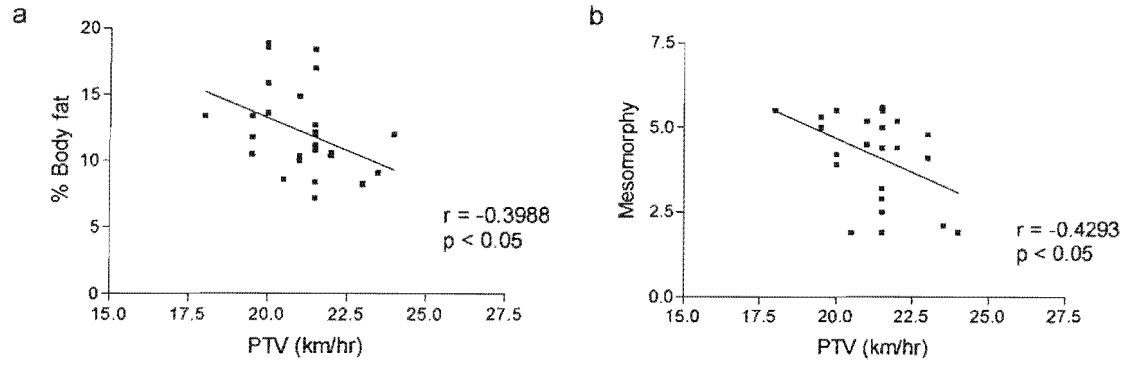


Figure 2.3: Correlation of peak treadmill velocity (PTV) with anthropometrical variables (n=27), namely: percentage body fat (a) and mesomorphy (b).

2.5.1.2 Oxygen consumption, heart rate and respiratory exchange ratio

The mean peak treadmill velocity (PTV) reached by the group of subjects during the maximal running test was 21.16 ± 1.32 km/hr (range 18 – 24 km/hr) and their peak heart rate (HR) was 188.76 ± 6.20 beats per minute (bpm). PTV, a laboratory-based measure of performance, was significantly negatively correlated with 10 km PB (r=-0.680, p<0.001, Figure 2.4), a field-based measure of performance.

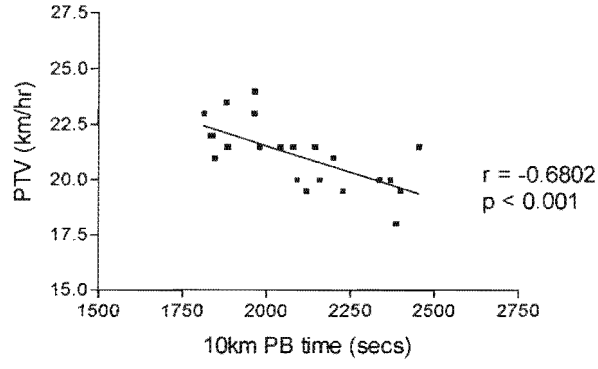


Figure 2.4: Correlation of peak treadmill velocity (PTV) with 10 km personal best time (PB) (n=24).

The subjects' resting, 50% of maximal and maximal VO_2 and RER data are shown in Table 2.3.

Table 2.3: Oxygen consumption (VO_2) and respiratory exchange ratio (RER) measured during rest (n=25), during running at 50% of peak treadmill velocity (PTV, n=23) and at the end of a maximal running test (peak; n=29). Values expressed as mean ± standard deviation.

	VO_2 (ml/kg/min)	RER
Resting	3.4 ± 1.2	0.85 ± 0.10
50% of PTV	39.5 ± 3.2	0.87 ± 0.04
Peak	67.7 ± 7.0	1.15 ± 0.06

VO_2max was significantly negatively correlated with the subjects' PB ($p<0.01$) and significantly positively correlated with the subjects' PTV ($p<0.01$, Figure 2.5 a and b). Neither peak HR nor peak RER correlated significantly with the subjects' PB or PTV (Figures 2.5 c, d, e and f).

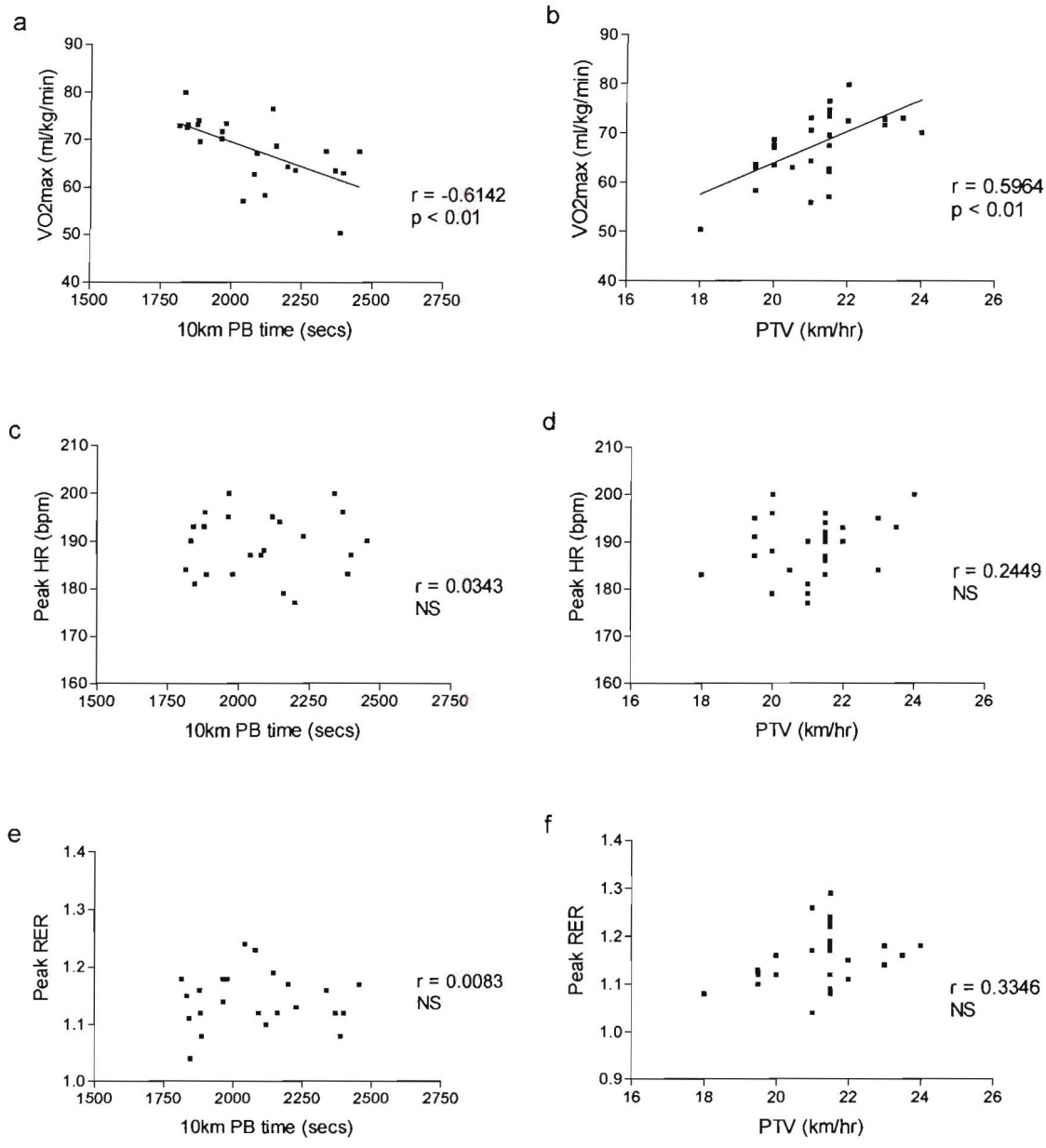


Figure 2.5: Correlation of performance measures with cardiorespiratory variables, namely: maximal oxygen consumption (VO_2max) with 10km personal best time (PB, a, n=24) and peak treadmill velocity (PTV, b, n=29), peak heart rate (HR) with 10km PB time (c, n=24) and PTV (d, n=29), and peak respiratory exchange ratio (RER) with 10km PB time (e, n=24) and PTV (f, n=28). bpm: beats per minute

The subjects' submaximal oxygen consumption, when expressed per kg of body mass, did not correlate significantly with their PB for any of the four absolute speeds in the interval running test (data not shown). However, when calculating running economy with

oxygen consumption expressed per $kg^{0.66}$ rather than per kg, oxygen consumption was correlated with PB at 12 ($p<0.01$), 14 ($p<0.05$) and 16 km/hr ($p<0.05$, Figure 2.6).

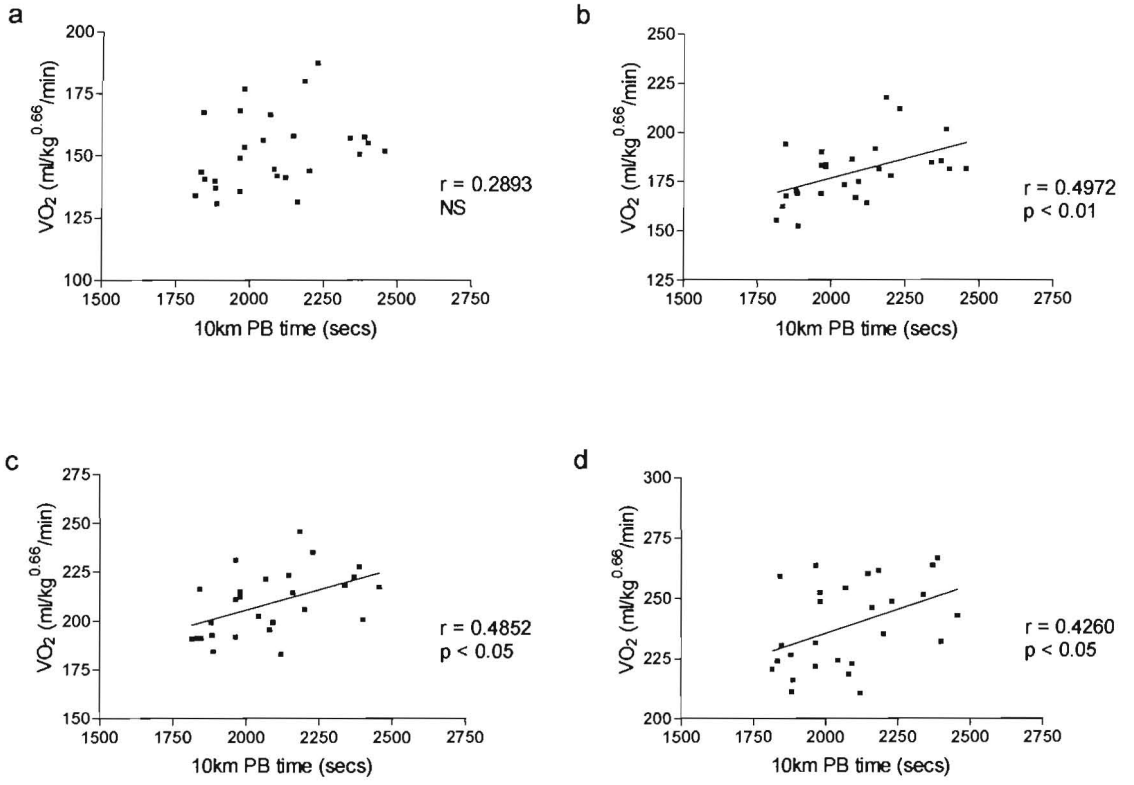


Figure 2.6: Correlation of 10km personal best time (PB) with running economy (n=27), namely oxygen consumption (VO_2) expressed per $kg^{0.66}$ (ml/$kg^{0.66}$/min) at 10 km/hr (a), 12 km/hr (b), 14 km/hr (c) and 16 km/hr (d).

2.5.1.3 Plasma metabolite concentrations

The subjects' resting, peak and 3 minutes post maximal running test plasma lactate, sodium and potassium concentrations are shown in Table 2.4.

Table 2.4: Resting, peak and 3 minutes post maximal running test plasma metabolite concentrations, namely: plasma lactate (resting n=31, peak n=26 and 3 min post n=27); plasma sodium (resting n=29, peak n=18 and 3 min post n=26) and plasma potassium (resting n=29, peak n=19 and 3 min post n=26) concentrations. Values expressed as mean ± standard deviation.

	Lactate (mmol/l)	Sodium (mmol/l)	Potassium (mmol/l)
Resting	1.5 ± 0.5	135.1 ± 1.6	4.18 ± 0.46
Peak	10.5 ± 2.8	139.3 ± 1.7	5.06 ± 0.55
3 min post	10.4 ± 3.0	137.1 ± 2.1	3.85 ± 0.35

Peak plasma lactate concentration was not significantly correlated with either the field-based or the lab-based measure of performance, namely PB and PTV (Figures 2.7 a and b). Peak plasma sodium and peak plasma potassium concentrations were also not significantly correlated with either PB or PTV, although there was a tendency towards a negative correlation between peak plasma potassium and PB ($p=0.051$, Figures 2.7 c, d, e and f).

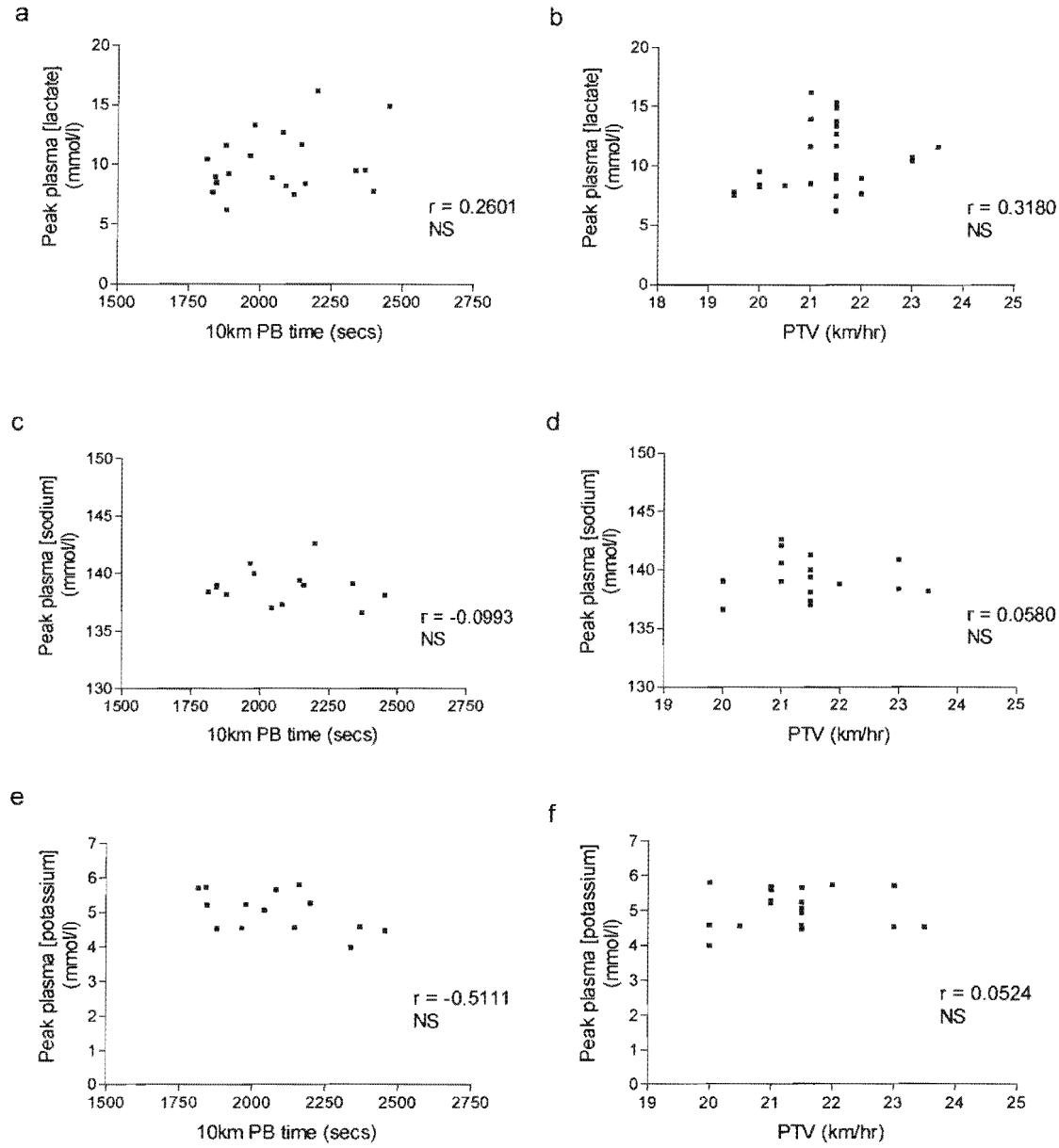


Figure 2.7: Correlation of performance measures with peak plasma metabolite concentrations, namely: peak plasma [lactate] with 10km personal best time (PB, a, n=21) and peak treadmill velocity (PTV, b, n=25), peak plasma [sodium] with 10km PB time (c, n=15) and PTV (d, n=17), and peak plasma [potassium] with 10km PB time (e, n=15) and PTV (f, n=18).

2.5.2 Part B: Ethnic comparison

2.5.2.1 Anthropometry

As shown in Table 2.5, the white runners were of significantly greater stature ($p<0.001$) and mass ($p<0.001$) than the black runners, and also had a greater lean body mass (LBM) value ($p<0.001$). Body mass index (BMI) and the SSS measurement were also significantly greater in the white runners ($p<0.05$), while mesomorphy ($p<0.01$) and percentage body muscle ($p<0.05$) were greater in the black runners. While there was no difference in LTV between the black and white runners, the black runners had a significantly higher LTV/LBM value ($p<0.01$), suggesting that their thighs were larger (more muscular) relative to the rest of their body than the white runners' thighs are. There was no significant difference between the black and white runners for either their endomorphy or ectomorphy values.

Table 2.5: Anthropometric variables for the black (n=16) and white (n=16) runners. Values expressed as mean ± standard deviation.

Anthropometric variable	Black	White
Age (yrs)	21.3 ± 5.7	24.6 ± 4.7
Height (cm)	168 ± 4	180 ± 8 ***
Weight (kg)	57.6 ± 6.4	72.2 ± 8.5 ***
BMI	20.5 ± 1.9	22.3 ± 1.9 *
LBM (kg)	51.0 ± 5.6	62.8 ± 7.4 ***
% Body fat	11.4 ± 2.1	13.0 ± 3.7
SSS (mm)	45.6 ± 8.0	56.5 ± 17.7 *
% Body muscle	56.4 ± 5.0	52.9 ± 4.5 *
LTV (cc)	3472 ± 376	3659 ± 719
LTV/LBM (cc/kg)	68.4 ± 7.4	58.2 ± 8.8 **
Endomorphy	2.04 ± 0.38	2.61 ± 1.13
Mesomorphy	5.00 ± 0.57	3.65 ± 1.39 **
Ectomorphy	3.28 ± 1.00	3.19 ± 0.93

BMI: body mass index; LBM: lean body mass; SSS: sum of seven skinfolds; LTV: lean thigh volume. *p<0.05; **p<0.01; ***p<0.001

The black runners' PB was positively correlated with their percentage body fat ($p<0.05$), SSS ($p<0.01$) and endomorphy ($p<0.05$, Figure 2.8 a, b and c), while their PTV was significantly correlated with their percentage body fat ($p<0.05$), SSS ($p<0.05$) and LTV/LBM value ($p<0.05$, Figure 2.8 c, d and e).

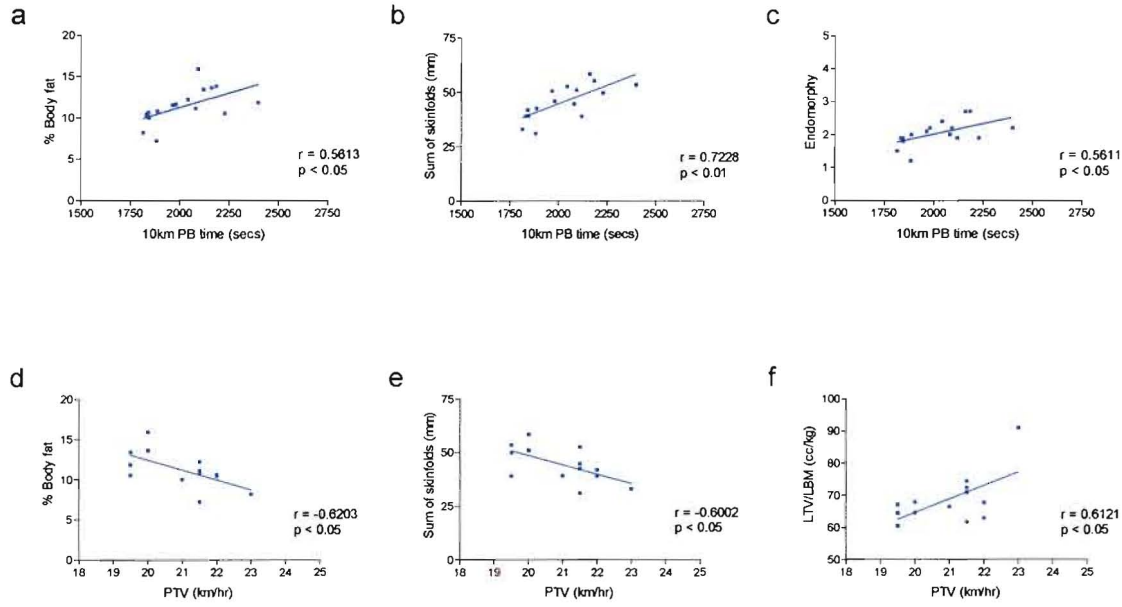


Figure 2.8: Correlation of 10km personal best time (PB) (n=16) and peak treadmill velocity (n=13) with anthropometrical variables in black runners, namely: PB with percentage body fat (a), sum of seven skinfolds (b) and endomorphy (c), and PTV with percentage body fat (d), sum of seven skinfolds (e) and lean thigh volume/lean body mass (f).

The white runners' PB correlated positively with their percentage body fat ($p<0.05$) and endomorphy ($p<0.05$, Figure 2.9 a and b), however there was no significant association between their PTV and any of their anthropometrical variables (data not shown).

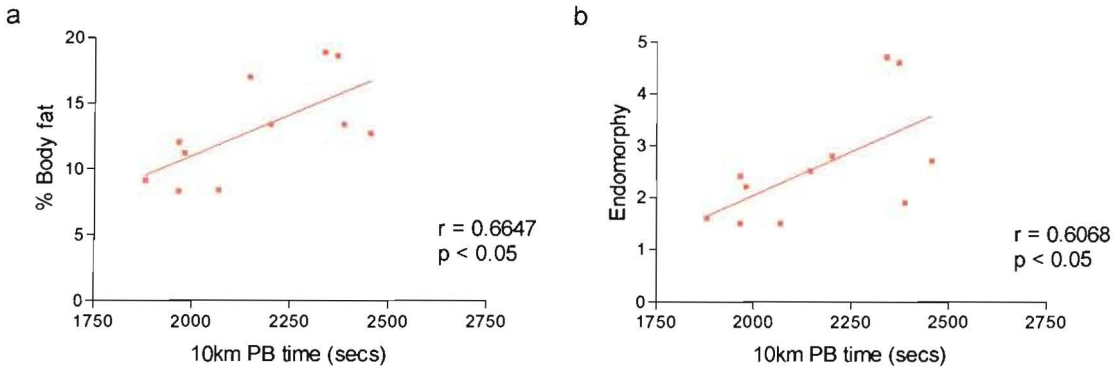


Figure 2.9: Correlation of 10km personal best time (PB) (n=11) with percentage body fat (a) and endomorphy (b) in white runners.

2.5.2.2 Oxygen consumption, heart rate and respiratory exchange ratio (resting, 50 % and peak)

There was no significant difference between the 10 km personal best times of the black (2023 ± 170 s or 33 min 43 s) and white (2159 ± 203 s or 35 min 59 s) runners. There was also no significant difference between the PTV reached by the black (20.96 ± 1.14 km/hr) and the white (21.31 ± 1.42 km/hr) runners or their peak HR (187.77 ± 5.20 and 189.56 ± 7.00 bpm) during the maximal treadmill running test. PTV was significantly negatively correlated with PB for both the black ($p<0.001$) and the white runners ($p<0.01$, Figures 2.7 a and b).

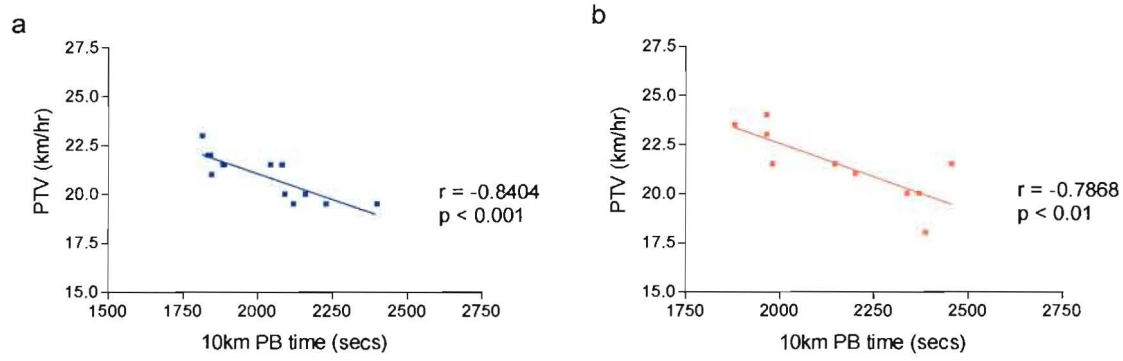


Figure 2.7: Correlation of peak treadmill velocity (PTV) with 10 km personal best time (PB) in black (a, n=13) and white (b, n=11) runners.

There were no significant differences between the black and white runners for VO_2 or RER values at rest, during the submaximal (50% of PTV) or maximal running tests (Table 2.6).

Table 2.6: Oxygen consumption (VO_2) and respiratory exchange ratio (RER) for black and white runners measured during rest (n=16 and 9, respectively), during running at 50% of peak treadmill velocity (PTV; n=13 and 10, respectively) and at the end of a maximal running test (peak; n=13 and 16, respectively). Values expressed as mean ± standard deviation.

		Black	White
VO_2 (ml/kg/min)	Resting	3.7 ± 1.1	2.9 ± 1.2
	50% of PTV	39.5 ± 3.2	39.6 ± 3.3
	Peak	67.9 ± 6.7	67.6 ± 7.4
RER	Resting	0.84 ± 0.09	0.87 ± 0.12
	50% cf PTV	0.86 ± 0.03	0.87 ± 0.05
	Peak	1.13 ± 0.06	1.17 ± 0.06

VO_2max correlated significantly with PB in both black ($p<0.01$) and white ($p<0.05$) runners, but with PTV only in white runners ($p<0.01$, Figure 2.10).

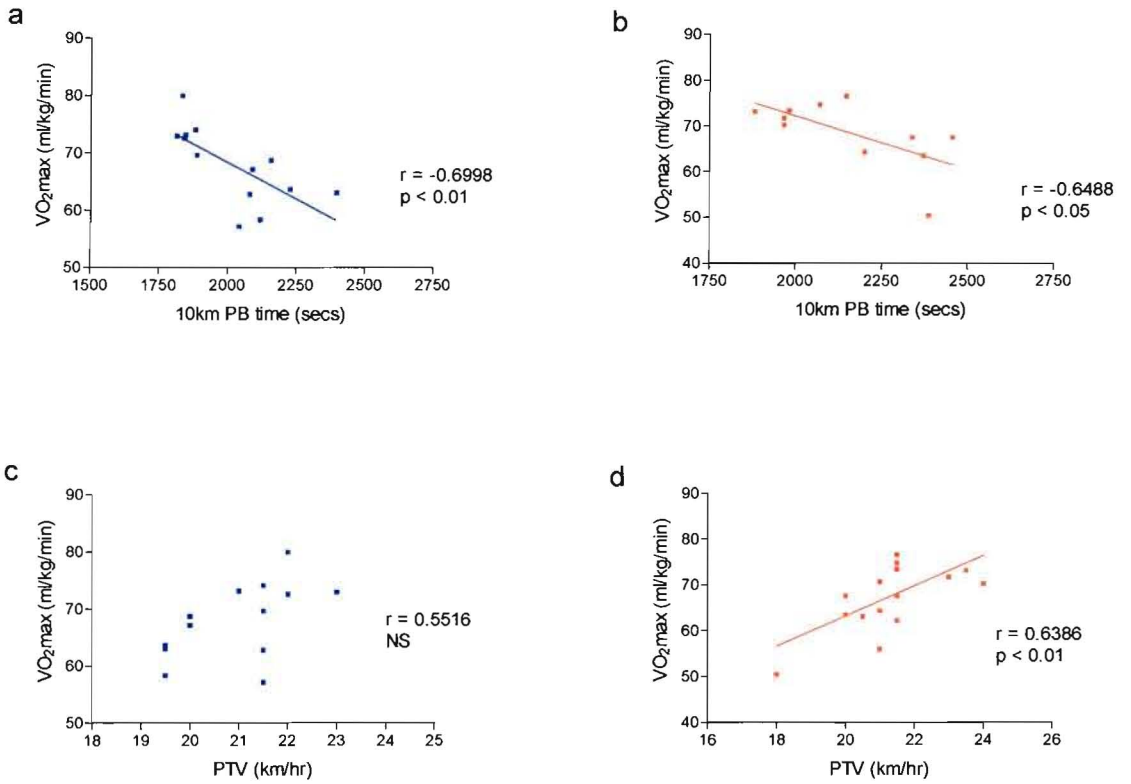


Figure 2.10: Correlation of maximal oxygen consumption (VO_2max) with performance measures, namely: 10km personal best time (PB) in black (a, n=13) and white (b, n=11) runners, and peak treadmill velocity (PTV) in black runners (c, n=13) and white (d, n=16) runners.

Unlike VO_2max, peak HR and peak RER did not correlate significantly with PB or PTV in either the black or the white runners (Figures 2.11 and 2.12).

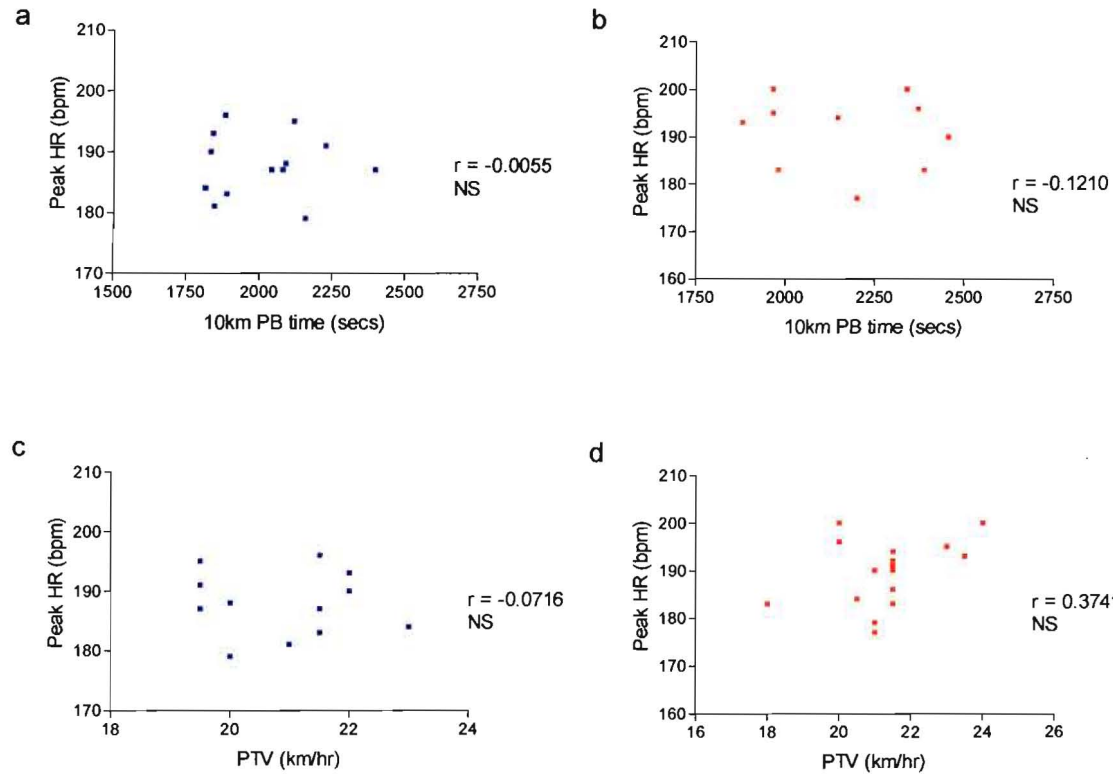


Figure 2.11: Correlation of peak heart rate (HR) with performance measures, namely: 10km personal best time (PB) in black (a, n=13) and white (b, n=11) runners, and peak treadmill velocity (PTV) in black runners (c, n=13) and white (d, n=16) runners. bpm: beats per minute

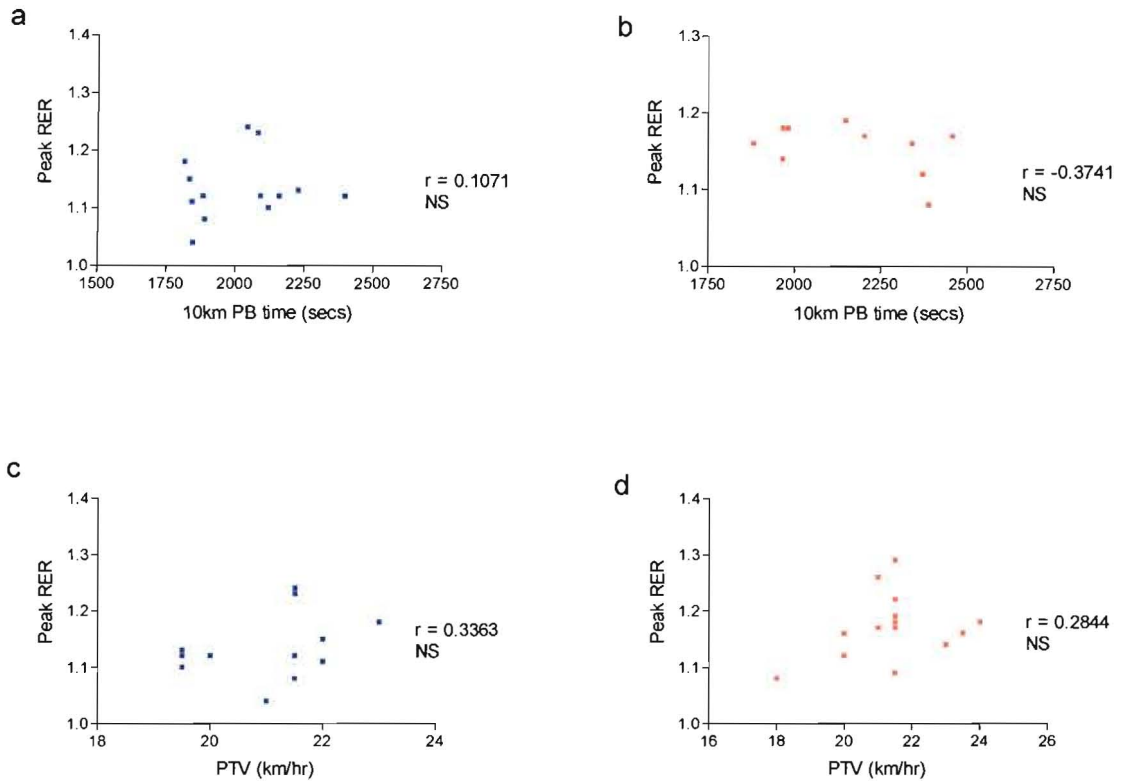


Figure 2.12: Correlation of peak respiratory exchange ratio (RER) with performance measures, namely: 10km personal best time (PB) in black (a, n=13) and white (b, n=11) runners, and peak treadmill velocity (PTV) in black runners (c, n=13) and white (d, n=15) runners.

2.5.2.3 Plasma metabolite concentrations (resting and peak)

Both the peak and the 3 min post maximal running test plasma lactate concentrations were significantly lower in the black than the white runners ($p<0.01$ and $p<0.001$, respectively, Table 2.7). While there was no significant difference between the black and the white runners for the peak plasma sodium concentration, the 3 min post sodium concentration was also significantly lower in the black runners compared to the white ($p<0.01$). The plasma potassium concentration, however, was significantly higher in the black runners than the white for the resting ($p<0.01$), peak ($p<0.01$) and 3 min post measurements ($p<0.001$).

Table 2.7: Resting, peak and 3 minutes post-maximal running test plasma lactate, sodium and potassium concentrations in black and white runners. Values expressed as mean ± standard deviation.

		Black	n value	White	n value
Lactate (mmol/l)	Resting	1.7 ± 0.6	16	1.4 ± 0.5	15
	Peak	8.7 ± 1.6	12	12.0 ± 2.7 **	14
	3 min post	8.3 ± 1.5	13	12.4 ± 2.8 ***	14
Sodium (mmol/l)	Resting	134.6 ± 1.1	15	135.7 ± 1.8	14
	Peak	138.3 ± 0.9	6	139.9 ± 1.8	12
	3 min post	136.0 ± 1.9	13	138.3 ± 1.5 **	13
Potassium (mmol/l)	Resting	4.40 ± 0.44	15	3.94 ± 0.37 **	14
	Peak	5.53 ± 0.31	6	4.84 ± 0.49 **	13
	3 min post	4.07 ± 0.33	13	3.63 ± 0.20 ***	13

*p<0.05; **p<0.01; ***p<0.001

Peak plasma lactate concentration, measured immediately after the maximal treadmill running test, did not correlate significantly with either PB or PTV in either the black or the white runners (Figure 2.13).

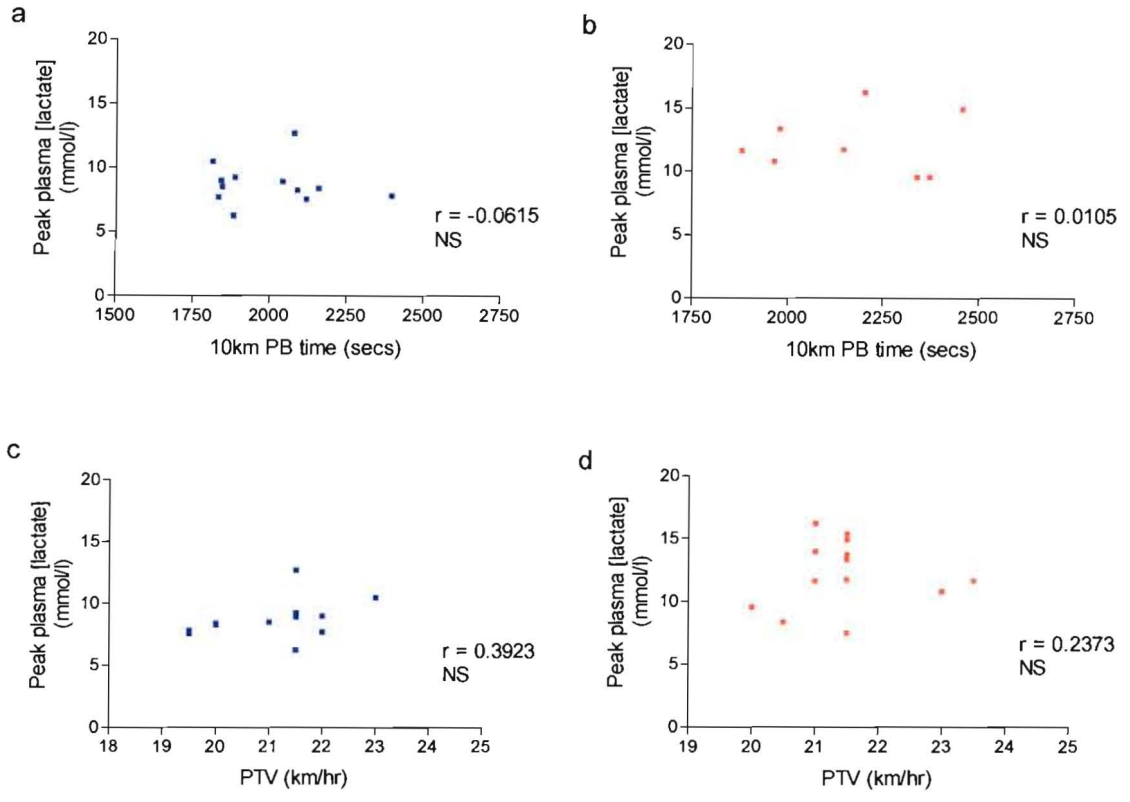


Figure 2.13: Correlation of peak plasma [lactate] with performance measures, namely: 10km personal best time (PB) in black (a, n=12) and white (b, n=9) runners, and peak treadmill velocity (PTV) in black runners (c, n=12) and white (d, n=13) runners.

The results were similar for peak plasma sodium and potassium concentration, which also did not correlate significantly with either PB or PTV in the black or the white group (Figures 2.14 and 2.15).

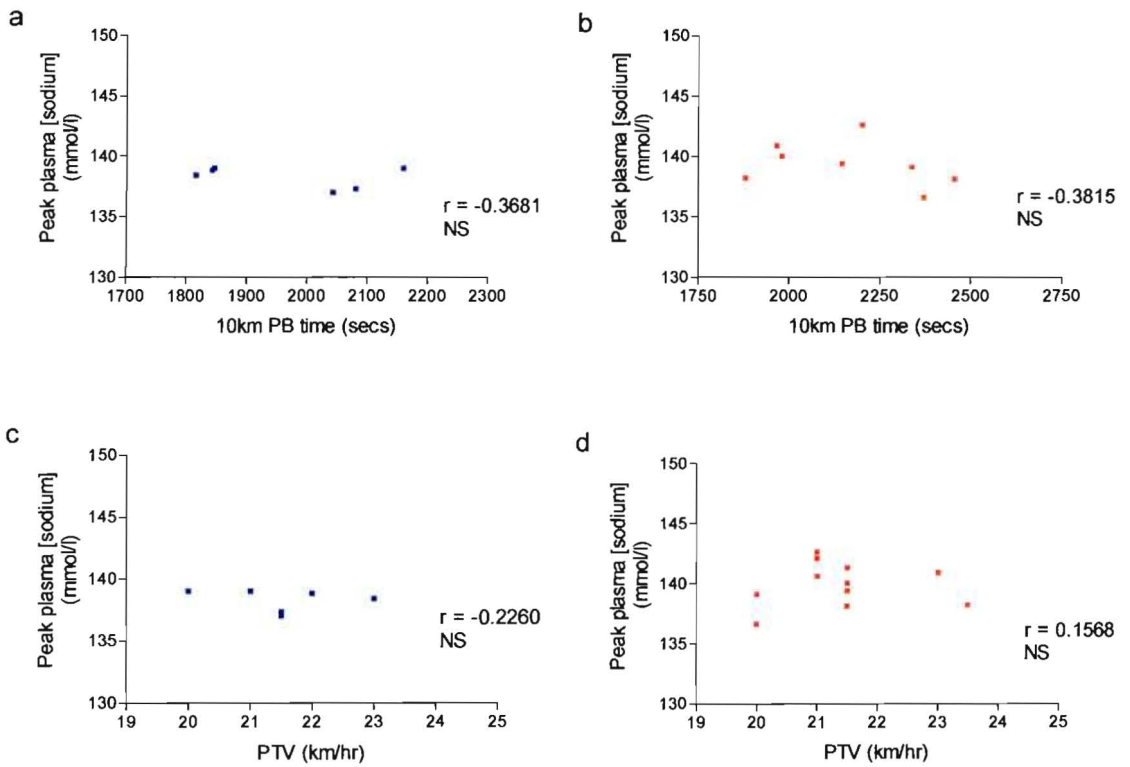


Figure 2.14: Correlation of peak plasma [sodium] with performance measures, namely: 10km personal best time (PB) in black (a, n=6) and white (b, n=9) runners, and peak treadmill velocity (PTV) in black runners (c, n=6) and white (d, n=11) runners.

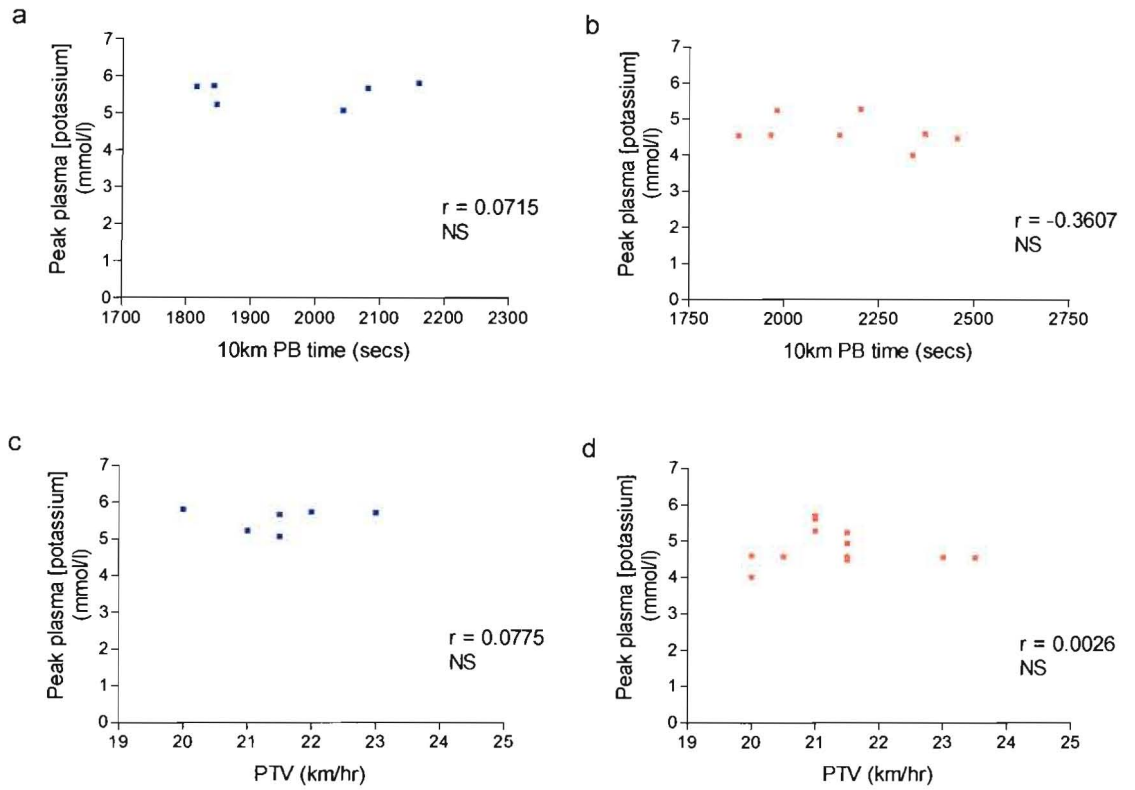


Figure 2.15: Correlation of peak plasma [potassium] with performance measures, namely: 10km personal best time (PB) in black (a, n=6) and white (b, n=9) runners, and peak treadmill velocity (PTV) in black runners (c, n=6) and white (d, n=12) runners.

2.5.2.4 Oxygen consumption, heart rate and respiratory exchange ratio (interval test)

There were no significant differences in submaximal oxygen consumption between the black and the white runners at any of the absolute speeds in the interval running test (Figure 2.16 a), indicating that there was no difference in running economy (VO_2 at a set absolute running speed) between the two groups. There were also no significant differences between the black and white runners for VO_2/%PTV at any of the four submaximal running intensities (Figures 2.16 b). However, when calculating running economy with oxygen consumption expressed per $kg^{0.66}$ rather than per kg, there was a significant difference in running economy between the two groups at 14 ($p<0.05$) and 16 km/hr ($p<0.01$, Figure 2.17).

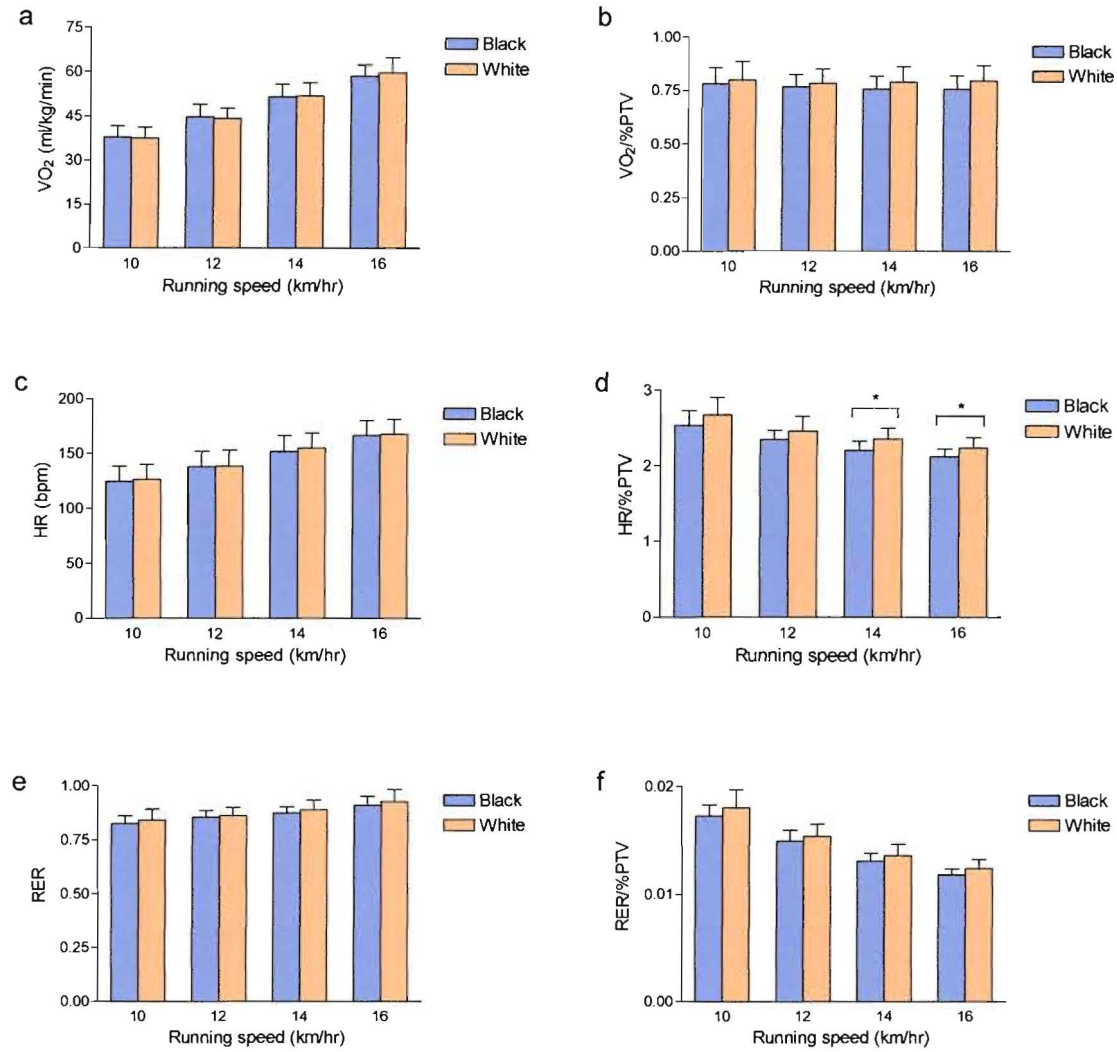


Figure 2.16: Cardiorespiratory variables of black and white runners at four speeds during an interval run: oxygen consumption (VO_2, a, black n=16; white n=13) and oxygen consumption per percentage of peak treadmill velocity (PTV, b, black n=13; white n=13); heart rate (HR, c, black n=16; white n=12) and heart rate per percentage of peak treadmill velocity (d, black n=13; white n=12); respiratory exchange ratio (RER, e, black n=16; white n=13) and respiratory exchange ratio per percentage of peak treadmill velocity (f, black n=13; white n=13). *$p<0.05$

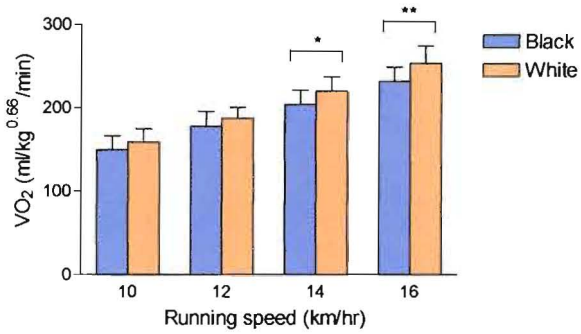


Figure 2.17: Oxygen consumption expressed per kg$^{0.66}$ (ml/kg$^{0.66}$/min) of black and white runners at four speeds during an interval run (black n=16; white n=13). *p<0.05; **p<0.01

There was no significant difference in mean heart rate between the black and white runners at any of the four absolute speeds during the interval running test (Figure 2.16 c). While there were no significant differences in HR/%PTV between black and white runners at the slower two interval test running speeds, the black runners' HR/%PTV was significantly lower than the white runners' at 14 (p<0.05) and 16 km/hr (p<0.05), indicating that the black runners' HR was lower when running at the same relative submaximal percentage of PTV as the white runners (Figure 2.16 d). There were no significant differences in RER or in RER/%PTV between the black and the white runners at any of the absolute speeds in the interval running test (Figure 2.16 e and f).

2.5.2.5 Plasma metabolite concentrations (interval test)

There were no significant differences in plasma lactate concentration between the black and white runners for the submaximal running intervals at 10, 12 or 14 km/hr (Figure 2.18 a and b). There was, however, a significant difference in plasma lactate concentration between the black and white runners both immediately after (p<0.05), and four minutes after (p<0.05), the 16 km/hr run, with the black runners' lactate concentration lower than that of the white runners. There were no significant differences in plasma [lactate]/%PTV between the black and white runners at 10 or 12 km/hr, but there were at 14 (p<0.05) and 16 km/hr (p<0.05) immediately post running and at 16 km/hr (p<0.05) four minutes post running, with the black runners' values again lower than that of the white runners (Figure 2.18 c and d).

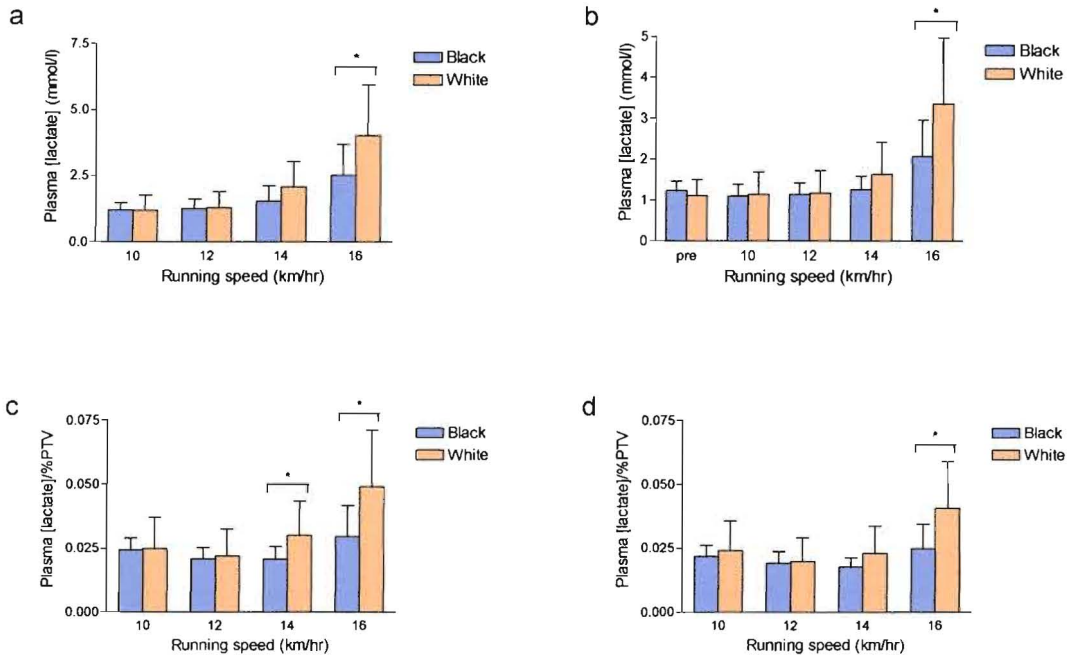


Figure 2.18: Plasma lactate concentrations of black and white runners during a submaximal interval running test: Plasma [lactate] (black n=16; white n=11) immediately post (a) and 4 minutes post (b) running; and plasma [lactate] per percentage of peak treadmill velocity (PTV, black n=13; white n=11) immediately post (c) and 4 minutes post (d) running. pre: blood sample taken immediately before interval running test. *p<0.05

There were no significant differences in plasma sodium concentration or in plasma [sodium]/%PTV between the black and white runners for any of the submaximal running speeds during the interval run (Figure 2.19).

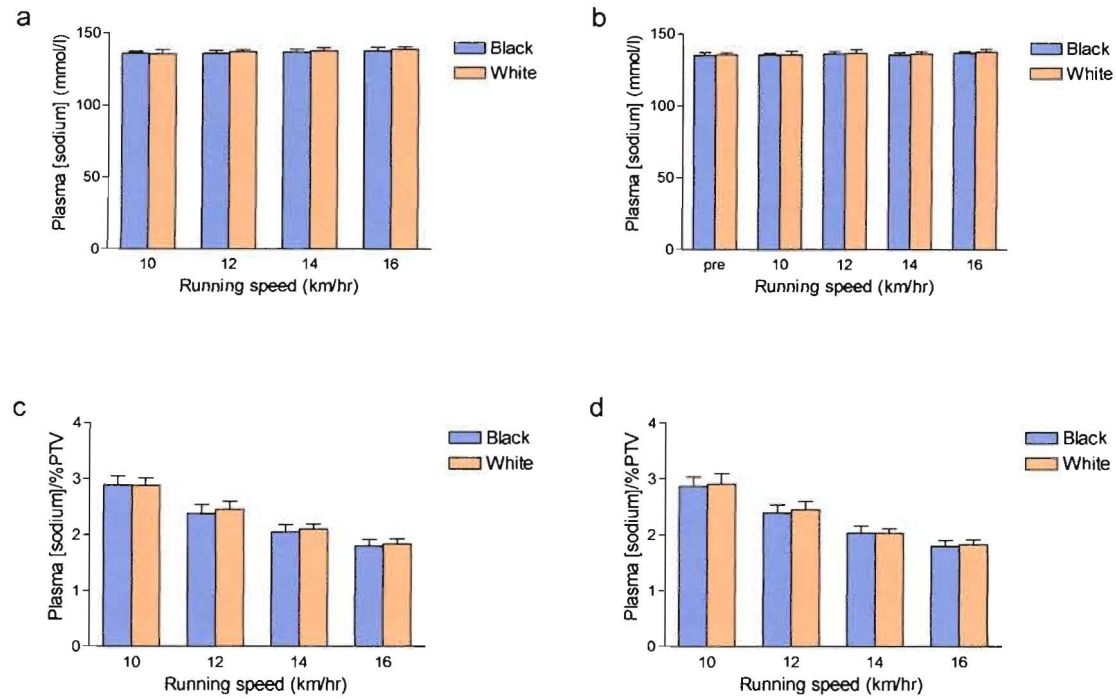


Figure 2.19: Plasma sodium concentrations of black and white runners during a submaximal interval running test: Plasma [sodium] (black n=14; white n=9) immediately post (a) and 4 minutes post (b) running; and plasma [sodium] per percentage of peak treadmill velocity (PTV, black n=11; white n=9) immediately post (c) and 4 minutes post (d) running. pre: blood sample taken immediately before interval running test.

Similar to plasma sodium, there were no significant differences in plasma potassium concentration or in plasma [potassium]/%PTV between the black and white runners for any of the submaximal running speeds (Figure 2.20).

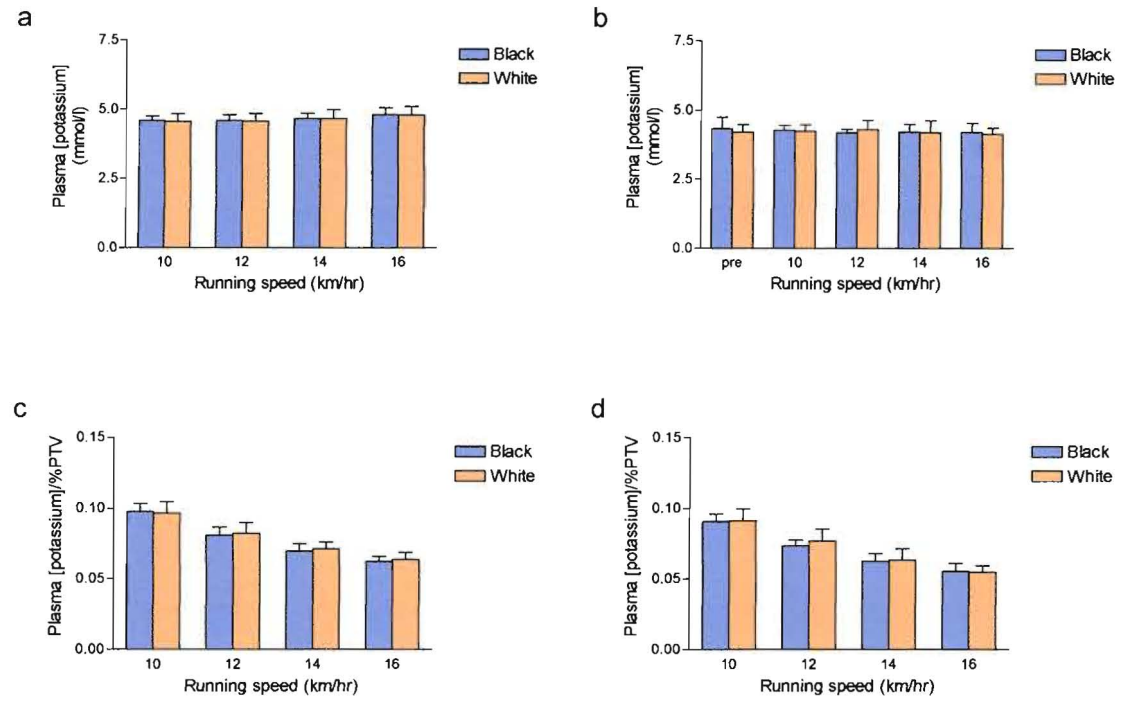


Figure 2.20: Plasma potassium concentrations of black and white runners during a submaximal interval running test: Plasma [potassium] (black n=14; white n=9) immediately post (a) and 4 minutes post (b) running; and plasma [potassium] per percentage of peak treadmill velocity (PTV, black n=11; white n=9) immediately post (c) and 4 minutes post (d) running. pre: blood sample taken immediately before interval running test.

2.6 DISCUSSION

2.6.1 Running economy

The findings of this chapter suggest a role for running economy in endurance performance as well as in the observed performance difference between black and white South African runners. A good running economy, in other words a low level of oxygen consumption at any particular exercise intensity, has been suggested to be beneficial to endurance running [89,319,339]. In support of this, running economy, with oxygen consumption expressed per $kg^{0.66}$, was positively correlated with 10 km running performance when measured at 12, 14 and 16 km/hr. The level of oxygen utilised at a certain exercise intensity indicates the metabolic cost to the individual performing the exercise. If one athlete has a lower metabolic cost than another athlete at a particular workload, he is exercising more economically than the other athlete. According to the results of this chapter, therefore, this greater economy during submaximal running is associated with superior race performance, at least over 10 km.

There was a significant difference between the running economy of the black and white South African runners, at 14 and 16 km/hr (with VO_2 expressed per $kg^{0.66}$). The black runners consumed less oxygen than the white runners when running at these higher speeds and hence were more economical. There may be many causes of this greater running economy in the black runners, including biomechanical differences, stride parameter differences, variations in neuromuscular recruitment or elastic energy utilisation, and differences in muscle metabolism, such as different uses of fuels or metabolic pathways. It should be noted that there are variations in the formula used to calculate running economy. For example, it has been calculated with VO_2 expressed per kg [84], per $kg^{0.66}$ [449] and per $kg^{0.75}$ [388]. This is due to the metabolic cost of exercise being affected by body mass [449]. When the running economy of the subjects in this thesis was calculated with VO_2 expressed per kg, there was no significant difference between the black and the white runners at any of the absolute speeds in the interval running test, and running economy did not correlate with 10 km PB. It is clear, therefore, that the formula used will influence the results, and this should be taken into account when comparing findings from different studies.

In a study of black and white elite South African runners matched for race performance, Coetzer et al [84] found no significant difference in running economy between the groups, with VO_2 expressed per kg. Weston et al [449] studied sub-elite black and white South African runners matched for both race time and body mass, and found that the black runners were significantly more economical than the white when running at 16.1 km/hr, whether VO_2 was calculated in ml/kg/min or ml/$kg^{0.66}$/min. The level of significance, however, was higher when VO_2 was expressed per $kg^{0.66}$. Saltin et al [388] found that the difference in oxygen consumption between Kenyan and Scandinavian runners when running at speeds from 10 to 16 km/hr was greater when VO_2 was expressed per $kg^{0.75}$ of body mass than per kg. Similar to the findings of this thesis, they found that the better running economy of the black African runners was more pronounced at higher running speeds.

2.6.2 Peak treadmill velocity

Of all the variables measured in this chapter, the one that correlated best with 10 km running performance was peak treadmill velocity, the laboratory-based measure of performance. This correlation was negative, implying that the higher the PTV a runner of this standard can reach, the quicker he can run a 10 km race. This relationship between PTV and field-based running performance is in agreement with previous research [339,399,448], and Noakes et al [339] also found that PTV was the best laboratory-based predictor of performance in 10 km races. PTV was also significantly negatively correlated with PB for both the black and the white South African runners, when analysed as separate groups. The correlation coefficients were actually higher when the two groups were analysed separately than when they were analysed together, and it is possible that the relationship between PTV and PB could be influenced by ethnicity. PTV was not, however, significantly different between the black and white South African runners. This is in agreement with the findings of Coetzer et al [84] and Weston et al [448], but different to the findings of Bosch et al [47], who found that black South African runners reached a significantly lower maximum workload (combination of speed and gradient) during a maximal running test than their white counterparts.

2.6.3 Maximal oxygen consumption, heart rate and respiratory exchange ratio

VO_2max was also significantly negatively correlated with PB, and significantly positively correlated with PTV. In other words, the greater the subjects' maximal level of oxygen consumption, the quicker they run a 10 km race and the faster speed they can reach during a maximal treadmill running test. VO_2max also correlated significantly with PB in the black and the white groups when analysed separately, hence maximal oxygen consumption is associated with running performance in both ethnic groups. VO_2max has been described as the main determinant, or one of the main determinants, of endurance performance [88,146,385,457]. However, VO_2max may be a better predictor of athletic performance when a heterogenous group of athletes is studied than when athletes of similar ability are evaluated [146,335]. Indeed, in contradiction to the results of this thesis, Weston et al [448] found no significant relationship between VO_2max and 10 km PB in a group of well-trained South African runners of a limited performance range. Noakes et al [339] reported that PTV was a better predictor of race performance than VO_2max was. The findings of this thesis are in agreement with this, although the difference between the correlation coefficients for the two analyses is slight ($r = -0.680$ for PTV and $r = -0.614$ for VO_2max).

As VO_2max is not perfectly correlated with PB, however, maximal oxygen consumption cannot be the only determinant of running performance, and other factors must play a role. It can of course be argued that an individual's VO_2max is the effect, rather than the cause, of his running performance [340]. In this case it is not the athlete's maximal rate of oxygen consumption that has allowed him (and limited him) to attain a PTV of 21 km/hr during a maximal treadmill test, but instead, by running at that speed, his oxygen consumption has merely increased to that level in response to the exercise. There are a multitude of physiological factors other than maximal oxygen consumption that could be involved in the fatigue experienced by an athlete and therefore his endurance performance ability.

Unlike VO_2max, however, neither peak HR nor peak RER correlated significantly with PB or PTV. This was the case when all the subjects' data were analysed together or when analysed as two separate ethnic groups, and it suggests that these variables are not associated with running performance. When comparing the ethnic groups, neither

VO_2max, nor peak HR, nor peak RER were significantly different between the groups. There was therefore no difference between the black and white runners in their maximal levels of oxygen consumption or their fuel utilisation when exercising maximally. Of the cardiorespiratory and muscular factors affecting VO_2max, the cardiorespiratory factors probably play a more limiting role, by limiting oxygen transport to the muscle [107,108,389]. It could therefore be argued that, as there was no difference between the two ethnic groups for VO_2max, there was presumably no difference in their rate of blood oxygen transport. However, this does not take into account factors that are not part of the cardiorspiratory system or the muscle, such as neuromuscular or central nervous factors, which are also likely to influence maximal oxygen consumption [340].

This finding that there was no difference between ethnic groups for VO_2max, peak HR and peak RER is mostly in agreement with previous research. Weston et al [448] reported no difference between black and white South African runners for peak HR or peak RER, although Coetzer et al [84] found peak RER was significantly lower in black runners than white runners, suggesting that the black runners were oxidising a relatively greater proportion of fats and lower proportion of carbohydrates than the white runners when exercising maximally. Bosch et al [47], Coetzer et al [84] and Weston et al [448] also found no differences in the VO_2max of black and white South African distance runners. Similar to this thesis, none of these studies matched the two ethnic groups for body size. While not for height, Weston et al [449], however, did match black and white South African runners for body mass and found VO_2max to be lower in the black athletes. This finding that the black athletes were able to achieve the same performance in 10 km races as their white counterparts despite having a lower maximal oxygen uptake, again suggests that VO_2max is not the only determinant of running performance, at least over 10 km. This finding also suggests that the similarity in VO_2max between black and white runners found in this thesis as well as by Bosch et al [47], Coetzer et al [84] and Weston et al [448], could be due to the subjects not being matched for body mass. As the study by Weston et al [449] is the only one to have matched for body mass, however, further research including an ethnic comparison with black and white runners matched for body mass is recommended before final conclusions regarding the relationship between VO_2max and ethnicity are drawn. Whether this finding of a lower VO_2max in black African athletes is real or not, however, it is unlikely to account for the observed superior performance of

black African athletes [338], considering that a low VO_2max is considered disadvantageous for endurance performance.

2.6.4 Submaximal heart rate and respiratory exchange ratio

Along with the finding that there was no ethnic difference in VO_2max or peak RER, there were also no significant differences between the black and white runners in VO_2 or RER at rest or during the submaximal (50% of PTV) running test. This indicates that there was no difference between the groups for oxygen consumption or fuel utilisation when at rest or when exercising at the same relative submaximal intensity. There were also no significant differences in RER or in RER/%PTV between the black and the white runners at any of the four speeds in the interval running test. This implies that the black runners were not using significantly different fuel sources (relatively more carbohydrate or more fat) to the white runners when running at the same absolute or relative submaximal intensity. In support of this data, Weston et al [448] also found no significant difference between black and white South African runners for RER at three different relative workloads. Bosch et al [47], however, found that black runners had a higher RER at submaximal speeds than white runners. This contradicts our findings, particularly as, although we found no significant difference, the RER for the black athletes is actually slightly lower than that for the white athletes at all four running speeds. The reason for the contradictory results could result from the runners in this thesis being tested in a fasted state, while Bosch et al [47] tested their subjects after instructing them to ingest carbohydrates "as they would before a race". In addition, the subjects in their study were matched for their marathon running ability, while the subjects in this thesis were matched for their 10 km running ability.

Similar to the RER results, there were no significant differences in submaximal HR between the black and the white runners at any of the absolute speeds in the interval running test. However, while there were no significant differences between the black and white runners in HR/%PTV when running at 10 and 12 km/hr, the black runners' values were significantly lower than those of the white runners at 14 and 16 km/hr. This means that their HR was lower than that of the white runners when running at the same relative high intensities. It is interesting to note that both VO_2 and HR were only lower in the black athletes at the higher running speeds, perhaps suggesting that any

cardiorespiratory differences between the ethnic groups are only evident at high exercise intensities. In contrast to these findings, Weston et al [448] reported no difference in HR between black and white runners running at three different relative workloads (72%, 80% and 88% of PTV), while Weston et al [449] reported that black South African runners running at 10 km race pace had a higher HR than white runners, when matched for body size.

2.6.5 Plasma metabolite concentrations

The peak plasma lactate, sodium and potassium concentrations were not significantly correlated with PB or PTV, both when the data was analysed for all the subjects together and when analysed for the two ethnic groups separately. Although not significant, there was a tendency towards a negative correlation of peak plasma potassium concentration with PB ($p=0.051$) when the subjects were analysed as one group, suggesting that a higher peak plasma potassium concentration could be associated with endurance performance. There is potassium efflux from the muscle with exercise, which is then eliminated from the blood by mechanisms including the Na^+/K^+ pump of the exercising muscle. Increased plasma potassium levels may both facilitate exercise and be involved in fatigue processes [301]. For example, potassium has been associated with stimulation of heart rate and ventilation [263,316] as well as vasodilation in contracting muscle [159], yet has also been suggested to decrease the strength of muscle contraction and add to the sensations of pain that can occur with prolonged exercise [263]. As a result it is not clear whether higher plasma potassium levels would be an advantage or a disadvantage to endurance performance.

2.6.5.1 Plasma lactate concentration

Both the peak and the 3 minutes post running plasma lactate concentrations measured after the maximal running test were significantly lower in the black South African runners than the white runners, although there was no significant difference between the resting values. The black runners' plasma lactate concentration decreased from peak to 3 minutes post, while the white runners' value increased, indicating that rate of plasma lactate clearance was greater than the rate of lactate accumulation during this period of recovery for the black runners, but the opposite for the white runners. There were no significant differences in plasma lactate concentration between the black and the white

runners for the submaximal running intervals at 10, 12 or 14 km/hr. There was, however, a significant difference in plasma lactate concentration between the two groups both immediately after, and four minutes after, the 16 km/hr run, with the black runners' lactate concentration again lower than that of the white runners. This indicates that the black athletes accumulated less plasma lactate than the white athletes when exercising at the same absolute high intensity. There were also no significant differences in lactate/%PTV between the black and white runners at the lower running speeds (10 or 12 km/hr), but there were at 14 and 16 km/hr immediately post running and at 16 km/hr four minutes post running, with the black runners' values again lower than that of the white runners. This indicates that the black runners' plasma lactate concentrations were lower than white runners' when running at the same relative, rather than absolute, intensities. This ethnic difference in exercising plasma lactate concentration therefore appears to be evident only at the higher exercise intensities, as indicated by the two groups' results being significantly different after running at 14 km/hr, 16 km/hr and after running maximally, but not after running at 10 km/hr, 12 km/hr or at rest.

This finding that black South African athletes have lower exercising plasma lactate concentrations than white athletes supports previous research. Bosch et al [47] reported lower blood lactate levels in black than white sub-elite South African marathon runners after a treadmill marathon, while Coetzer et al [84] found elite black middle- to long-distance runners had lower blood lactate concentrations than white runners after running at 21 km/hr and after a maximal treadmill test. Weston et al [448] also reported that sub-elite black South African runners had lower plasma lactate levels than white runners after running at a high submaximal intensity (88% of PTV), and found that the black runners accumulated lactate at a significantly slower rate with increasing exercise intensity. This ethnic difference is not restricted to South African athletes. Saltin et al [388] found that blood lactate concentrations were lower in elite Kenyan than Scandinavian runners at a given submaximal exercise intensity. However, they found no differences between the groups for peak blood lactate values.

The implication of variations in blood lactate concentration in response to exercise is extremely complex [66]. The lower plasma lactate concentrations in the black runners at the higher running intensities could be a result of a difference between the two ethnic groups in the rate of lactate production, the rate of lactate transport between the muscle

and the blood, as well as the rate of lactate oxidation by the muscle and other tissues [84]. It is suggested that ethnic differences in lactate flux rates and blood lactate clearance be investigated in the future. In addition, plasma lactate accumulation during exercise is also affected by the non-steady-state lactate distribution volume, which is smaller in lighter athletes [101]. The rate of lactate production will depend on the rate of glycogenolysis and glycolysis occurring in the muscle as well as the rate of lactate formation from pyruvate (facilitated by the protein lactate dehydrogenase (LDH)). These factors will in turn depend on the fuel utilisation, with an increase in the accumulation of lactate reflecting increased utilisation of carbohydrates [66]. It is therefore possible that the activity of the glycogenolytic and glycolytic pathways or the concentration of LDH is different between the black and white runners during high intensity running, causing lactate to accumulate slower.

The transport of lactate between the muscle and the blood is mediated by the sarcolemmal monocarboxylate transporter (MCT) proteins [63]. These proteins are also responsible for transporting lactate and pyruvate across the mitochondrial membrane for entry into the citric acid cycle and subsequent oxidative respiration [59]. It is therefore also possible that a difference in the activity of, or the sarcolemmal and/or mitochondrial content of, MCT's between the black and white runners could result in the lower plasma lactate concentration in the black athletes with exercise. These possibilities will be discussed further in the Intramuscular factors chapter (Chapter 3) of this thesis.

A relevant question is whether or not the differences in exercising plasma lactate between black and white distance runners is related to the observed difference in performance between these ethnic groups. There is more than one way in which lactate can affect fatigue and endurance performance. The lactate levels in the cytosol must be prevented from rising to too great an extent for high rates of glycolysis to be maintained, as is necessary during exercise. In addition, according to the cell-cell and intracellular lactate shuttle models, lactate can be taken up by skeletal muscle and used as an oxidative fuel [59]. Therefore lactate can act as a respiratory fuel, aiding energy replacement during exercise. As lactate and H^+ are transported across the sarcolemmal membrane together, lactate transport out of the cell is also of importance in muscle pH regulation, especially during exercise when lactate flux is high, as the cotransporter is driven more by the lactate gradient than pH. Ethnic differences in muscle and blood

lactate levels may therefore also be related to differences in muscle pH. Muscle H^+ accumulation may contribute to fatigue during exercise [142,302,382]. The flux of lactate between the muscle and blood may therefore affect the development of fatigue, and hence performance during endurance exercise, by influencing the rate of glycolysis, by altering the supply of lactate as a respiratory fuel and by regulating the muscle pH. If these metabolic process are in some way different between black and white South African runners, then the observed plasma lactate concentration differences between these ethnic groups may indeed be associated with their performance difference.

2.6.5.2 Plasma sodium concentration

While there were no significant differences between the black and the white runners for the resting or peak plasma sodium concentrations, a novel finding of this thesis is that the 3 minutes post maximal running test sodium concentration was significantly lower in the black runners compared to the white runners. Sweat rate and post-exercise body mass were not measured, therefore any differences in the degree of sweating between the two groups could not be determined. As the difference between the groups was evident 3 minutes post running when the plasma sodium concentration had decreased from the peak value, this could suggest a faster recovery of plasma sodium in the black compared to the white runners, possibly due to faster re-uptake of sodium by the muscle. If this were the case it could suggest a difference in the expression or activity of the Na^+/K^+ pumps between the ethnic groups. A lower activity of, or a reduction in the muscle concentration of, Na^+/K^+ pumps can result in a decrease in muscle excitability [82]. Therefore, a difference in the functioning of these pumps can affect muscle force output and therefore performance. During the interval running test, the black runners' plasma sodium concentration values were consistently lower than those of the white runners, but these differences were not significant between the two groups for any of the four submaximal running speeds. Similarly, there were no significant differences between the two groups at any of the running speeds for plasma [sodium]/%PTV. Therefore, there are not any consistent differences in exercising plasma sodium concentrations between the black and white runners, and the validity of the significant difference found at 3 minutes post maximal running would need to be confirmed with further research. In addition, investigation of the muscle concentrations of Na^+/K^+ pumps in the two ethnic groups is recommended.

2.6.5.3 Plasma potassium concentration

Another novel finding was that the plasma potassium concentration was significantly higher in the black runners than the white runners for the resting, peak and 3 minutes post running measurements. While this could result from greater muscle activity, and therefore greater efflux of potassium from the muscle, by the black runners compared to the white runners during exercise, this would not explain the difference in the resting measurement. The increased plasma potassium levels with exercise may be involved in both the facilitation of exercise as well as in the development of fatigue, as described earlier [301]. As a result it is not clear whether the higher plasma potassium levels evident in the black athletes would be an advantage or a disadvantage to endurance performance. In both the black and the white runners the 3 minutes post running plasma potassium value after the maximal running test was lower than the resting value. This post-exercise potassium concentration undershoot compared to resting levels can be explained by a higher gain of the Na^+/K^+ pump after exercise [306]. For both the black and the white runners, and in agreement with previous research [306], the plasma potassium concentration increased with increasing exercise intensity during the interval running test. Despite the ethnic difference in peak plasma potassium concentration, however, there were no significant differences in plasma potassium concentrations between the black and the white runners for any of the four submaximal running speeds during the interval running test. There were also no significant differences between the two groups for plasma [potassium]/%PTV at any of the four running speeds. It is not clear why the ethnic difference in plasma potassium concentration was evident during rest and after maximal exercise, but not during submaximal running. Further studies examining the ethnic difference in plasma and muscle potassium flux during exercise of varying intensities is therefore needed.

2.6.6 <u>The effect of exercise intensity on ethnic differences</u>

It is interesting to note that many of the differences between the black and the white runners were only apparent at the higher exercise intensities, for example running economy (with VO_2 in $ml/kg^{0.66}/min$) at 14 and 16 km/hr; HR/%PTV at 14 and 16 km/hr; plasma [lactate] at 16 km/hr and at maximal; plasma [lactate]/%PTV at 14 and 16 km/hr; and plasma [potassium] at maximal. These differences therefore seem to be apparent when running at roughly sub four minutes a kilometer (15 km/hr). The subjects for this

chapter were chosen based on their ability to run a 10 km race at roughly 4 minutes a kilometer or less (a personal best 10 km time of under 41 min). The physiological differences between the ethnic groups would therefore be relevant for runners of this caliber and above, as they are performing at these high intensities in competition. This phenomenon of ethnic physiological differences at high exercise intensities may not be restricted to a South African population. Saltin et al [388], for example, found that elite Kenyan runners had a better running economy than Scandinavian runners at high, but not low running speeds.

2.6.7 Anthropometry

Tanaka and Matsuura [427] suggested that anthropometric variables could predict distance running performance to approximately the same degree as cardiorespiratory factors. In this thesis, percentage body fat, SSS, endomorphy, LTV and LTV/LBM were all significantly correlated with 10 km PB. The anthropometrical variables that displayed the highest level of significance were those related to body fat content, namely percentage body fat and SSS. These correlations were positive, suggesting that the lower a runner's level of body fat, the faster he would run a 10 km race, which is consistent with previous research [16]. Berg et al [22] reported that somatotype and body mass index can explain a moderate portion of the variance in 10 km run performance in moderately-trained runners who are heterogeneous in ability. However, this is likely to be less in a more homogeneous group of well-trained runners, such as those in this thesis. Analysis of the two ethnic groups separately revealed that similar anthropometrical characteristics were associated with 10 km running performance in both groups. The black runners' 10 km PB was positively correlated with their percentage body fat, SSS and endomorphy, suggesting again that low levels of body fat are linked to endurance performance. The black runners' PTV correlated significantly with percentage body fat, SSS and LTV/LBM. This last variable suggests that muscular thighs relative to the rest of the body could be an advantage in a maximal running test, possibly due to the leg power required for the high speeds reached during this test. In the white group, percentage body fat and endomorphy correlated positively with PB, suggesting that, similar to the black runners, body fat content is to related to 10 km running performance.

The white runners were of significantly greater stature and mass than the black runners, and also had a greater LBM value. This finding confirms similar reports from previous studies of black and white South African distance runners [47,84,448], and appears to be the case for sedentary black and white South African men as well [114]. A smaller body mass may be advantageous during endurance activity, as the rise body temperature can be a limiting factor during exercise [102,226,283]. Marino et al [285] found that heat storage was positively correlated with body mass at 35 °C, only moderately correlated at 25 °C, and no correlation was evident at 15 °C. They suggested that, compared to heavier runners, lighter runners produce and store less heat at the same running speed and hence can run faster or further before reaching a limiting rectal temperature. They concluded that runners with a lower body mass have a thermal advantage when running in conditions in which heat-dissipation mechanisms are being maximally utilised. The runners in this chapter were running in a thermoneutral environment in the laboratory (maintained at 22 °C) with a fan blowing on them, which provided additional cooling. Potential advantages for the smaller black runners over the larger white runners that could result from running in a hot environment were therefore minimised. Marino et al [284] studied highly trained African and Caucasian runners exercising in hot and cool environments. They found that, while the Africans ran faster than the Caucasians (8km time trial) in the heated condition (35 °C), the two groups ran a similar time in the cool condition (15 °C). This suggests that thermal advantages resulting from the smaller body mass of the black runners compared to the white is unlikely to account for differences in performances between these groups during cool conditions. It therefore also suggests that thermal differences are probably not likely to account for any ethnic differences recorded in the laboratory during subject testing for this thesis.

Mesomorphy and percentage body muscle were greater in the black runners than the white runners. Despite this, BMI was significantly greater in the white runners, perhaps due to their higher body fat content, which was significant for the SSS measurement, but not for the percentage body fat calculation. It should be acknowledged that there can be differences in the density of the fat-free mass in different ethnic groups which can affect body fat equations [334], as described in the methods. However, the lower SSS in the black runners could confer an advantage for them over the white runners in endurance activities. Coetzer et al [84] also found that black South African runners had a lower SSS than their white counterparts, while Weston et al [448] reported that black and white

runners had a similar percentage body fat. Bosch et al [47] noted a difference in fat patterning between black and white South African runners, with the black athletes having a lower skinfold thickness for thigh, calf and triceps. Along with the greater mesomorphy value and percentage body muscle, the black runners in this chapter also had a significantly higher LTV/LBM value than the white runners, suggesting that their thighs were larger or more muscular relative to the rest of their body than the white runners' thighs were. It is possible that this could be an advantage during high intensity running due to the production of a large muscle power output relative to body size.

Therefore, while not a part of the cardiorespiratory system, the anthropometrical phenotype of a distance runner is associated with performance in endurance running. There are also differences in anthropometrical characteristics between black and white South African distance runners, which could be related to the observed differences in their distance running performance ability. Similar to cardiorespiratory and other physiological factors therefore, anthropometrical factors are important in the study of fatigue and endurance performance.

2.7 CONCLUSION

The findings of this chapter are summarised in Figure 2.21. Running economy at 12, 14 and 16 km/hr was positively correlated with 10 km running performance, when VO_2 was expressed per $kg^{0.66}$ of body mass. In addition, there were significant differences in running economy between the black and white South African runners at 14 and 16 km/hr, when VO_2 was expressed per $kg^{0.66}$ of body mass, with the black runners being more economical than the white. Peak treadmill velocity correlated with 10 km running performance slightly better than VO_2max did, whilst peak HR and peak RER showed no significant correlation with performance. There were no significant differences between the black and white runners in PTV, VO_2max, peak HR or peak RER. There were also no significant differences in submaximal HR or RER between the black and the white runners when running at 10, 12, 14 or 16 km/hr. As expected from previous research, the anthropometric variables related to body fat content were significantly associated with running performance, but peak plasma concentrations of lactate, sodium and potassium were not. The black athletes were shorter and lighter than the white athletes, with a lower sum of skinfolds value, yet with a higher mesomorphy score and a greater percentage body muscle. Both the peak and the 3 minutes post running plasma lactate concentrations measured after the maximal running test were significantly lower in the black athletes than the white. The plasma lactate concentration was also lower in the black runners than the white after running submaximally at 16 km/hr. While we found no significant difference between the black and the white runners for the peak plasma sodium concentration, a novel finding of this thesis was that the 3 minutes post running sodium concentration was significantly lower in the black runners compared to the white. Another novel finding was that the plasma potassium concentration was significantly higher in the black runners than the white for both the peak and the 3 minutes post running measurements. There were, however, no significant differences in plasma sodium or potassium concentration between the black and the white runners for any of the four submaximal running speeds. Many of the physiological differences between the black and the white runners were only apparent at the higher exercise intensities, which would be relevant during competition, when the athletes are performing at these high intensities. Therefore, in summary, while PTV and VO_2max were significantly correlated with 10 km running performance, there was no difference in these variables between black and white South African runners. Peak plasma concentrations of lactate and

potassium were different between the two groups, but these variables were not significantly correlated with running performance. Running economy and plasma lactate concentration were both, however, different in the black and white athletes when running at a high intensity as would occur during a race, and running economy was also correlated with 10 km running performance. It would therefore be useful to investigate the causes of the differences in these factors between the ethnic groups.

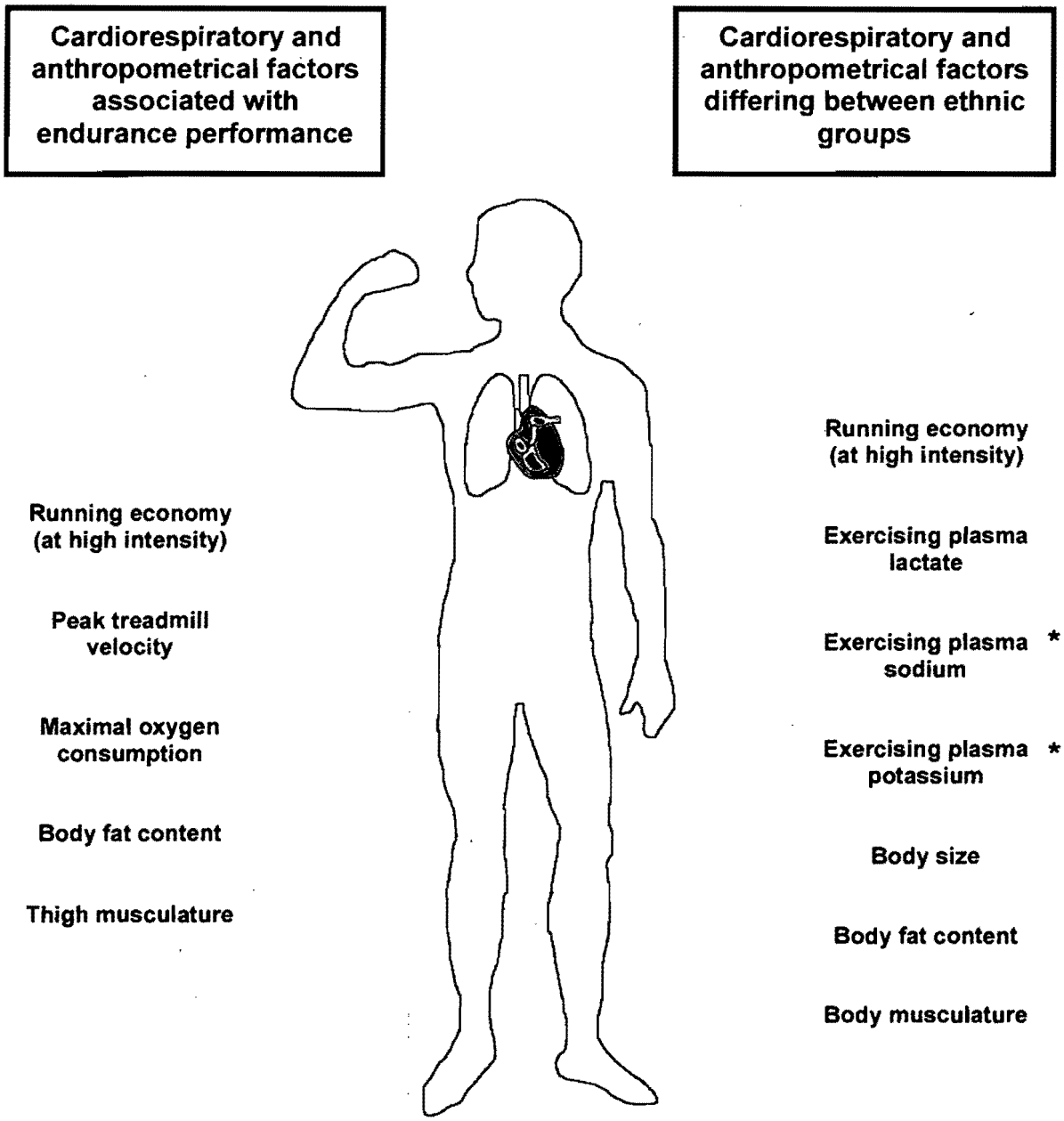


Figure 2.21: Summary of the cardiorespiratory and anthropometrical factors measured in this chapter that are associated with endurance performance and/or are different between black and white South African runners. * indicates novel findings

Chapter 3 Intramuscular factors

3.1 PREAMBLE

One of the main findings of the previous chapter (Chapter 2) was that both peak plasma lactate concentration and plasma lactate concentration when running at high submaximal intensities was lower in the black South African runners compared to the white South African runners. This is consistent with previous research on black African runners and is an unexplained metabolic phenomenon [47,84,388,448]. Lactate flux in the muscle and blood may affect the development of fatigue, and hence performance during endurance exercise, by influencing the rate of glycolysis, by altering the supply of lactate as a respiratory fuel and by regulating the muscle pH. Lactate has therefore been studied fairly extensively in the exercise sciences, although there is still disagreement as to the exact nature of the role played by lactate in fatigue processes.

The ethnic difference in exercising plasma lactate concentration could result from a difference in glycolytic pathway activity in black compared to white athletes. This in turn may be due to the ethnic groups exercising at different relative intensities or metabolising different relative proportions of carbohydrates and fats as fuels. The latter is perhaps unlikely, as there was no difference in RER at any exercise intensity between the black and white runners, as described in the previous chapter (Chapter 2). In addition, the ethnic differences in plasma lactate levels during exercise could also result from differences between the groups in oxidative pathway activity involving the citric acid cycle or the electron transport chain. This would result in different mitochondrial or cytosolic levels of pyruvate and, via the action of lactate dehydrogenase (LDH), different levels of lactate. As the activities of the glycolytic and oxidative pathways differ between the various muscle fibre types, unequal proportions of the fibre types between the ethnic groups could translate to differences in the total muscle or blood lactate flux. Lastly, the ethnic discrepancy in plasma lactate could also result from a difference in the efficiency of lactate transport between the muscle and the blood, or from the cytosol into the mitochondria for oxidation. These potential transport differences could be related to ethnic variations in muscle monocarboxylate transporter (MCT) density.

Aside from their potential ethnic disparities, skeletal muscle fibre composition and MCT content both play a role in endurance performance due to their functions in oxidative metabolism. A greater type 1 fibre proportion or lactate transporter content could delay the development of fatigue through multiple mechanisms. Therefore, these intramuscular factors are relevant for further investigation, and will be examined in this thesis chapter.

It should be noted that the term 'intramuscular', as used in this chapter title, has been employed to distinguish the physiological factors examined in this chapter from those of the Neuromuscular factors chapter (Chapter 4). In the context of this thesis, therefore, 'intramuscular' refers to all parts of the muscle cell, including the sarcolemma, but excluding neural control factors.

3.2 LITERATURE REVIEW: The role of intramuscular factors in fatigue and endurance performance

Contraction of skeletal muscle allows movement of the human body and hence physical activity. Skeletal muscle has a large capacity for adaptation [386], both during acute exercise and with training. Muscle fibres are recruited during exercise in a manner dependent on the type and intensity of the exercise and an appropriate metabolic response is generated by the muscle contraction. Low intensity exercise involves a relatively small level of muscle recruitment, while high intensity exercise results in increased recruitment, particularly of type 2 muscle fibres, and increased sympathetic nervous system activity [66]. The metabolism of fuels to produce energy for muscle contraction also changes depending on the intensity of the exercise. Carbohydrates, lipids and, to a lesser degree, proteins are oxidised in the muscle to produce energy in the form of ATP [66]. This fuel utilisation involves activity of metabolic pathways such as glycolysis, lypolysis, the citric acid cycle and the electron transport chain. During exercise, energy is produced from carbohydrate and lipid oxidation in varying proportions depending on exercise intensity as well as the genotype and training status of the individual involved [59,66]. Lipids predominate as an energy source during rest and low exercise intensities, while carbohydrates predominate at higher exercise intensities [66]. Many physiological factors can affect the level of the fuel sources and hence energy pathways used in the muscle with exercise, such as the level of activity of the sympathetic nervous system, the size of the carbohydrate and lipid stores in the body, blood flow distribution, blood lactate level, muscle recruitment and muscle mitochondrial mass [66].

With the fatigue that occurs during prolonged exercise, there are changes in the concentrations of metabolites that are associated with muscle contraction [290]. These changes can affect the fatigue process either directly within the muscle or via afferents to the spine and brain. Changes in the muscular concentrations of metabolites such as calcium, hydrogen, lactate, sodium and potassium can affect the contractile process [142,290]. Fatigue reduces Ca^{2+} release, Ca^{2+} uptake and Ca^{2+} ATPase activity in the sarcoplasmic reticulum [260]. An altered intracellular calcium regulation therefore appears to contribute to the reduced muscle force with fatigue via depressed sarcoplasmic reticulum function [260].

Changes in hydrogen ion concentration in the muscle during exercise are related to the changes in lactate concentration, with both of these increasing with fatigue [56,264,290]. The resulting reduction in intracellular pH decreases the excitability of the muscle fibre membrane [290]. Changes in the intra- and extracellular concentrations of sodium and potassium with fatigue alter the gradient of potentials across the sarcolemma, and therefore affect the conduction of excitation along the muscle fibre [290]. All of these factors can affect the muscular contractile processes such that muscle fatigue is accompanied by a decrease in maximal muscle tension or force output, as well as a reduced power and shortening velocity [142].

Many different physiological factors change in the muscle with endurance training, and these changes confer a performance advantage to the athlete. Muscle fibre composition changes with training [196] and the capillary supply to the muscle increases [204]. Muscle volume density of both mitochondria and intracellular lipid increases with endurance training [196]. An increase in mitochondrial protein concentration, and therefore oxidative enzyme concentration, occurs with endurance training [173]. Similarly, the concentration of muscle monocarboxylate transporter proteins also increases [113]. All of these training-induced changes result in an increased oxidative capacity of the muscle, allowing for a greater aerobic energy metabolism [196]. This adaptability of skeletal muscle is therefore important in endurance performance [386].

3.2.1 Fibre type

Many of the physiological and biochemical properties of human skeletal muscle fibres are determined by the motor nerves that innervate them, and in particular by the firing frequency of the innervating nerve [117,356,392]. The contractile properties of muscle fibres within a motor unit are therefore similar [65]. Most muscles in humans are composed of a mixture of fibre types [329], with fibres roughly grouped into type 1 and type 2 fibres.

3.2.1.1 Fibre classification

Type 1 and type 2 muscle fibres have, amongst many other protein isoforms, different isoforms of myosin heavy chains, troponin components and tropomyosin [33,34,106]. Type 1 fibres have the slow isoforms and type 2 fibres the fast isoforms, and therefore these

fibre types are also commonly referred to as slow twitch and fast twitch fibres, respectively. Muscle fibres are generally classified according to their myosin heavy chain isoforms [51,355]. Various methods are used to identify the relative fibre composition of a muscle, including histochemical techniques and electrophoretic separation techniques [394]. The most common technique is that of histological myofibrillar ATPase staining, with type 1 and type 2 fibres demonstrating low and high ATPase activity with staining, respectively [65].

The type 2 fibre classification is generally categorised into type 2A fibres and type 2B fibres using standard histological ATPase staining. Mitochondrial volume density is generally highest in type 1 fibres, lower in type 2A fibres and lowest in type 2B fibres [196,204]. Capillary supply is also greatest to the type 1 fibres, lower in type 2A fibres and lowest in type 2B fibres [204]. Considering their low mitochondrial density and capillary supply, type 2B fibres are low in oxidative capacity, while type 1 and type 2A fibres are high in oxidative capacity [65]. Combining knowledge of the fibres' contractile and oxidative capacity indicates that type 1 fibres are slow, oxidative fibres; type 2A are fast, oxidative-glycolytic fibres; and type 2B are fast, glycolytic fibres [65]. The myosin heavy chain isoforms determined using electrophoretic techniques are generally present as unique isoforms in the histochemically defined 'pure' fibre types [431]. However, there also appear to be rare muscle fibres that show properties of both type 1 and type 2 fibres, or properties inbetween those of type 2A and type 2B fibres, and different myosin heavy chain isoforms may coexist in these fibres. Histochemical staining has revealed a range of these fibres, which vary in their properties, and have been described as a group as myofibrillar ATPase intermediate fibres [392]. One of the most commonly described of these intermediate fibres are the so-called type 2C fibres, and it is possible that type 2C fibres are a transitional form of fibre between type 1 and type 2A fibres [392]. In addition, a type 2X fibre has been described using electropheretic and immunoblotting techniques, although this fibre can be equated to the type 2B fibre determined histologically in human muscle [65,181]. As the different fibre typing techniques identify different components of the fibre, the full range of possible fibre types and their relative similarities to each other are still not entirely clear.

3.2.1.2 Fibre type, power and endurance

Type 2 muscle fibres generally have greater diameters than type 1 fibres and therefore a faster conduction velocity, although this is not always the case [130,159]. Type 2 fibres are also capable of producing a greater force output than type 1 fibres [50,51] and are therefore important in the production of large force outputs. Indeed, Komi and Tesch [241] found that individuals with muscles made up of a high proportion of type 2 fibres demonstrated higher peak knee extension torque than did individuals with muscles made up of a high proportion of type 1 fibres. The velocity of contraction also differs relative to muscle fibre type proportion, such that individuals with higher percentages of type 2 fibres have been found to have a faster rate of force development [309,428]. A greater proportion of type 2 fibres has also been shown to be associated with a greater rise of the body's center of gravity during a squat jump, suggesting a type 2 fibre proportion is related to the ability to produce a greater power output [309].

While a high proportion of type 2 muscle fibres is advantageous for strength and power activities, a greater proportion of type 1 fibres is advantageous for endurance activities [338]. Many studies have shown that the extent of fatigue during endurance activities is related to fibre composition, such that individuals with a high proportion of type 2 muscle fibres are more susceptible to fatigue than those with a high proportion of type 1 fibres [241,260,271,434]. Muscle fibre composition is therefore often considered one of the main factors involved in determining endurance capacity [72]. Taylor et al [428], however, found no correlation between time to fatigue during sustained isometric knee extension and fibre type. Maughan et al [292] similarly found that isometric endurance capacity in untrained subjects was not correlated with muscle fibre composition and suggested that isometric endurance may not be influenced by muscle fibre composition in untrained individuals. A study investigating dynamic exercise performance found that fibre type proportion correlated with VO_2max in well-trained subjects, but not in sedentary subjects [272], again suggesting that the relationship between muscle fibre composition and endurance performance may depend on the training status of the individuals tested.

Skeletal muscle fibre composition varies to a large extent between individuals [292,404]. In general, there is a relatively equal proportion of type 1 and type 2 fibres in sedentary people [292,329]. Endurance trained athletes, however, often have a higher percentage of type 1 fibres in their skeletal muscle than untrained individuals or less trained athletes

[136,204,257,260]. Endurance trained athletes have also been shown to have a higher proportion of type 1 fibres and a lower proportion of type 2A fibres than resistance trained individuals [260]. Fink et al [136] contend that, while fibre composition is not a good predictor of distance running ability when making a comparison within a group of elite distance runners, the percentage of type 1 fibres can be used to discriminate between good and elite distance runners. Burke et al [70], however, found no difference between elite cyclists and competitive trained, but not elite, cyclists for percentage of type 1 or type 2 fibres, as well as the area of the different fibre types.

Skeletal muscle fibre composition can be determined by both genetic and environmental factors, with probably about 45 % of the variance in the proportion of type 1 fibres associated with inherited factors [404]. The large proportion of type 1 muscle fibres generally found in endurance athletes could therefore result from genetic endowment or training [196]. It is not clear how great a role each of these factors play in determining the muscle fibre composition in endurance athletes, as the athletes are generally only tested once they are already at the trained level. If these athletes had inherited a high proportion of type 1 fibres, it is possible that they could have selected to partake in endurance sport as a result of the aerobic advantages their fibre composition may have offered them.

3.2.1.3 Fibre type and training

Studies of the plasticity of muscle fibre composition with endurance training have yielded inconclusive results. Some studies have induced changes in fibre composition via chronic electrical stimulation of the muscle [383,432], while others have investigated changes after long-term exercise protocols [14,196,204,392,405]. Both endurance training and high-intensity intermittent training have been shown to increase the percentage of type 1 fibres and decrease the percentage of type 2B fibres in skeletal muscle [196,405]. In contrast, other studies have found no change in fibre type distribution pattern after training [14,148,348]. Schantz and Dhoot [392] found that, with endurance training, the proportion of myofibrillar ATPase intermediate fibres (including type 2C fibres) increased and the proportion of type 2 fibres decreased. The authors concluded that it was likely that type 2 fibres were converted into intermediate fibres with training, and that these intermediate fibres were a temporary fibre form in the transition of type 2 to type 1 fibres.

The stimuli and mechanisms involved in changing the expression of type 1 and type 2 fibres, however, is not yet clear.

Training also affects factors related to muscle fibres other than simply the fibre types' relative proportions. Ingjer et al [204] found that endurance training increased the capillary supply of all fibre types in the quadriceps muscle. Endurance training has also been shown to increase the mitochondrial volume density in type 1, type 2A and type 2B fibres [196], which the authors concluded would result in improved oxidative capacity of all the muscle fibre types. Howald et al [196] also found that the volume density of intracellular lipid increased after training, which they suggested could be associated with an increased ability to metabolise lipids for energy production. Increased utilisation of lipids during endurance exercise would have a muscle glycogen sparing effect and could thereby enhance performance.

3.2.1.4 Fibre type and ethnicity

As muscle fibre composition is affected by an individual's genotype, it is possible that the proportions of the fibre types are different in different ethnic populations. Ama et al [8] found that sedentary black subjects of West and Central African origin had a greater proportion of type 2A fibres and a lower proportion of type 1 fibres than sedentary white subjects. The black subjects also had greater enzyme activities than the white subjects for enzymes involved in anaerobic energy pathways, suggesting that the black individuals were suited to sporting events of short duration. As black Kenyan distance runners are known to perform well internationally, Saltin [384] compared the percentages of type 1 and type 2 muscle fibres as well as the cross-sectional area of the fibre types in untrained Kenyan boys and Danish boys, but found no significant difference. Similarly, no difference was found in fibre composition between Kenyan and Scandinavian runners [387]. Only two studies have investigated fibre composition in endurance athletes from different South African populations (Table 2.1, Chapter 2). These studies reported no difference in fibre type percentages in either elite or sub-elite black and white South African runners [84,448].

As mentioned previously, the concentrations of muscle proteins in the different fibre types vary. Certain isoforms of the monocarboxylate transporter family of proteins are found in skeletal muscle, and vary in concentration depending on fibre type and the

individual. These proteins facilitate lactate transport across the cell membrane and their role in exercise, and in fatigue during endurance exercise will now be discussed.

3.2.2 Monocarboxylate transporters and lactate transport

Lactate production via the glycolytic pathway increases from resting levels with physical activity. However, as well as producing lactate, working muscle is a major site of lactate clearance during exercise [418]. Exercising human skeletal muscle is therefore capable of simultaneous lactate production and removal [373,418]. For many years lactate has been considered by exercise scientists to be simply the end product of oxygen-limited metabolism during exercise. Contrary to this popular belief, studies have shown that glycolytic flux in muscle with exercise involves lactate formation and utilisation under aerobic conditions [86,418]. Brooks [58,59] suggests that lactate represents an important means of distributing carbohydrate potential energy for oxidation and gluconeogenesis. This premise is linked to the theories of the 'cell-cell lactate shuttle' and the 'intracellular lactate shuttle', which hold that lactate is a key intermediate in fuel utilisation during exercise [59]. Another recent theory that re-examines skeletal muscle energetics is that of the 'glycogen shunt' [403]. This hypothesis involves the continual and rapid regeneration of glycogen during muscle contraction, with lactate serving as a buffer between fast and slow energy needs [403], and this theory warrants further investigation.

3.2.2.1 Lactate shuttles

According to the cell-cell lactate shuttle (previously termed the intercellular lactate shuttle) hypothesis, lactate formed in muscle cells with high rates of glycogenolysis and glycolysis becomes an energy source and a gluconeogenic precursor at other sites [59]. Lactate produced by glycolytic muscle fibres could therefore be consumed by more oxidative fibres. The cell-cell lactate shuttle does not only operate between skeletal muscle cells, it also functions between the cells of tissues with net lactate release and cells from tissues where gluconeogenesis occurs, for example between the skeletal muscle and the liver [62]. The intracellular lactate shuttle, in contrast, describes the uptake and oxidation of cytosolic lactate by the mitochondria within the cell [59]. Glycolysis followed by oxidation of lactate to pyruvate within the mitochondria of a muscle cell allows a high glycolytic flux rate as well as maintaining redox balance in the cytosol and the mitochondria [62].

Lactate therefore functions as an advantageous metabolic intermediate between carbohydrate storage forms (glucose and glycogen) and metabolic end products (CO_2 and H_2O) [415]. The advantage of lactate as a metabolic intermediate is that it is rapidly exchanged between tissue compartments. With glycolysis during physical activity, glycolytic fibres accumulate and release lactate as a result of their low mitochondrial content, while oxidative fibres act as lactate sinks because of their greater mitochondrial density [64]. Glycolytic and oxidative activity in different muscle cells are therefore linked by lactate, which may be formed in one cell and used as a substrate in another.

As the production and utilisation of lactate occurs not only in muscle, but in multiple tissues, lactate forms a connection between these different tissues. Lactate is therefore involved in whole body metabolism and metabolic communication between tissues [179]. There is evidence of lactate shuttles functioning in tissues other than muscle. Pellerin et al [353] have described an astrocyte-neuron lactate shuttle, and recent work by McClelland et al [297] indicates the likely presence of a peroxisomal lactate shuttle, which supports the theory of lactate/pyruvate exchange across membranes of organelles or cells for the purpose of maintaining redox balance. Lactate is therefore no longer considered simply as a metabolic waste product, but as an important substrate involved in energy metabolism in the heart, liver, skeletal muscle and even brain [366].

3.2.2.2 The monocarboxylate transporter family

Lactate and other monocarboxylates are transported across the cell membrane and other membranes by a family of integral membrane transport proteins known as monocarboxylate transporters, or MCT's. The MCT transporter family consists of at least eight different isoforms (MCT1-MCT8) [119,212] of between 40 and 60 kDa [41]. It is thought that all isoforms have twelve transmembrane-spanning helices with intracellular C- and N-termini and a large intracellular loop between transmembrane-spanning segments six and seven [212].

The various MCT's isoforms have different properties, with different specificities for inhibitors and for the transport of moncarboxylates. They have varying affinities for importing or exporting the different monocarboxylates and may favour different cotransport cations [367]. The MCT isoforms have also been found to display different

cellular location and tissue specificities and to have varying inter-species distributions [71]. MCT's have been found in many different species, from rats, mice and hamsters to chickens, pigs and humans [63,73,98,160-162,206,207,238,254,262,297,358,367,400,458]. MCT isoforms occur in many different organs and tissues in the body. They are expressed in the skeletal muscle, heart, blood, kidneys, liver, brain, intestine and testes [15,63,149,160,161].

3.2.2.3 MCT's and skeletal muscle

As a result of their role in transporting lactate across membranes, MCT's form a fundamental part of the lactate shuttle models. MCT-facilitated lactate transport in skeletal muscles is quantitatively important in the regulation of whole body lactate metabolism [298], with skeletal muscle MCT's needing to support the high rates of flux of lactate in muscle with exercise [363]. Lactate production in the muscle increases from resting levels with exercise, and the lactate needs to be transported out of the cytosol in order to allow the continuation of glycolysis. At physiological pH, lactic acid is dissociated almost entirely into lactate anions and protons [400], and lactate transport across the sarcolemma occurs down proton and lactate concentration gradients [62]. MCT-mediated transport of lactate across the cell membrane therefore plays a central role in regulating the turnover of lactate in skeletal muscle.

Lactate flux in the muscle is, however, also regulated by mitochondrial lactate uptake and oxidation [61]. MCT's have been found not only in the sarcolemma, but also in the muscle mitochondrial membrane [71]. The presence of mitochondrial LDH [64,330] suggests that muscle mitochondria oxidise lactate [63,425]. It is therefore likely that mitochondrial LDH and a mitochondrial MCT together facilitate the transport of lactate into the mitochondria, and the subsequent transformation of lactate into pyruvate for oxidation. Both lactate and pyruvate can be transported from the cytosol into the mitochondria by MCT's. However, as muscle cytosolic LDH readily reduces pyruvate into lactate [59], there are much greater cytosolic concentrations of lactate than pyruvate, especially during exercise, and so lactate is more abundant for transportation [71]. Lactate is more reduced than pyruvate and can therefore, in combination with pyruvate, affect cell or organelle redox balance [297]. As a result, in addition to regulating lactate flux and providing substrates for mitochondrial oxidation, MCT's and LDH function to maintain redox balance in the cellular compartments in skeletal muscle [297].

3.2.2.4 MCT1 and MCT4

The MCT isoforms 1, 2 and 4 all occur in skeletal muscle, with MCT1 and MCT4 being the two major isoforms expressed in human skeletal muscle [212]. MCT2 has a high affinity for pyruvate and is expressed in various tissues in the body, although its expression in skeletal muscle is weak [212,262,364]. MCT1 is present in many tissues, with its expression in the heart, in skeletal muscle and in red blood cells particularly strong [15,60,161]. MCT4 also has a fairly broad tissue distribution, but is particularly strongly expressed in skeletal muscle and is the only MCT expressed in white blood cells [367,454]. MCT1 and MCT4 interact with CD147 [234], a cell membrane glycoprotein, and it is thought that this accessory protein allows their proper localisation to the sarcolemma [119,234,455].

MCT1 and MCT4 appear to have different roles in skeletal muscle [43]. MCT1 content is higher in type 1 than type 2 muscle fibres, and higher in type 2A than type 2B fibres [140,298]. MCT1 content therefore correlates positively with the percentage of type 1 fibres or the percentage of oxidative fibres in skeletal muscle [298,361]. MCT1 content likewise correlates positively with citrate synthase activity, a marker of the oxidative capacity of muscle [113,298], as well as with lactate uptake in skeletal muscle [298]. In contrast, MCT1 correlates negatively with the percentage of type 2 glycolytic fibres in skeletal muscle [43], as well as with total LDH activity, an index of a muscle's glycolytic capacity [298]. This suggests that MCT1 is related to the oxidative nature of a muscle, allowing more lactate to be taken up into oxidative muscle fibres, with a high mitochondrial content, than glycolytic fibres, so that it may be used in oxidative metabolism. MCT's catalyse monocarboxylate transport in both directions across membranes, dependent on the intra- and extracellular monocarboxylate concentrations [454]. Therefore, while MCT1 transports lactate both into and out of skeletal muscle cells, it is possibly asymmetric in function, acting predominantly as a lactate influx (as opposed to efflux) transporter [73,364].

The interindividual differences in MCT4 content in muscle have been found to be much larger than the differences in MCT1 content [113,361]. MCT4 content has been found to be low in type 1 fibres and high in type 2 fibres [140,361,454], and has also been found to correlate positively with the percentage of type 2 glycolytic fibres in skeletal muscle [43]. It is believed that MCT4 is probably the major MCT isoform responsible for glycolytically derived lactate efflux from (mainly type 2) muscle fibres [282,454]. MCT4 is suited for transporting lactate from glycolytic muscle fibres, especially during exercise, because it

has a high capacity, is non-saturable and preferentially transports lactate over pyruvate under physiological conditions [110].

MCT1 is found in sarcolemmal membranes as well as in mitochondrial membranes, while MCT4 has only been found in sarcolemmal membranes [63,71,113]. This suggests that MCT1 could play a role in the hypothesised cell-cell and intracellular lactate shuttles, with MCT4 only involved in the cell-cell shuttle [63,113]. It would therefore follow that sarcolemmal MCT4 (and to a lesser degree MCT1) accelerates efflux of lactate during exercise from glycolytic fibres to either neighbouring oxidative fibres or the blood for disposal. Sarcolemmal MCT1 would also facilitate the uptake of lactate from the blood into oxidative fibres. Mitochondrial MCT1, in turn, would facilitate lactate uptake (and hence oxidation) by the mitochondria of oxidative fibres both during and after exercise [113]. This channeling of the lactate produced by type 2 fibres with exercise to type 1 fibres for oxidation could help to minimise or delay fatigue [140].

3.2.2.5 The effect of training on MCT's

An increase in MCT content with training could reduce intramuscular lactate concentrations during exercise by facilitating the extrusion of lactate out of muscle [41]. Lactate transport capacity in the muscle does indeed appear to be related to the training status of the muscle [360]. Pilegaard et al [359] investigated a group of people with a wide range of fitness levels and found them to have widely ranging differences in lactate transport capacity, with sarcolemmal lactate transport capacity higher in athletes than in untrained and less trained subjects. Training has been shown to improve the lactate transport capacity of skeletal muscle in rats [299] and increase the peak muscle lactate release in humans [215]. The increase in sarcolemmal lactate transport with training could result from an increase in MCT density.

Indeed, skeletal muscle MCT1 and MCT4 content have been shown to increase with training in rats, although the changes were dependent on the muscle investigated as well as the intensity of the training [15,123,128]. MCT1 and MCT4 content have also been shown to increase in the skeletal muscle of humans after bicycle training [42,113,175], high-intensity knee-extensor training [215,360] and strength training [214]. The expression of MCT's in muscle is therefore plastic and responsive to different forms of training. Pilegaard et al [360] found that the increases in MCT content were associated with an increase in

sarcolemmal lactate transport, and noted that MCT1 density increased more than MCT4. Dubouchaud et al [113] similarly found that skeletal muscle MCT1 content increased to a greater extent than MCT4 content, and reported that while MCT1 content increased in total muscle preparations, sarcolemma-enriched and mitochondria-enriched fractions, MCT4 content increased significantly only in the sarcolemma-enriched fraction (and was indeed not found in the mitochondria-enriched fraction).

Interestingly, neither skeletal muscle MCT1 nor MCT4 content increased after high-intensity training, and MCT1 actually decreased after moderate-intensity training, in already highly trained athletes [126]. This suggests that the relative change in MCT density with training is dependent on the training status of the individual and the intensity of the training. Despite the possibility of changes in MCT content occurring rapidly with training [175], an acute bout of non-exhaustive exercise was found to produce no alteration in MCT1 content in rat skeletal muscle [127]. It is possible that acute muscle contraction increases the intrinsic activity of MCT's rather than increasing their content in the cell membrane [435]. Chronic electrical muscle stimulation appears to increase the expression of MCT1, but not MCT4 [44,454], while denervation results in decreased expression of both MCT1 and MCT4 [454]. The signal for the change in MCT expression with training is not yet known, however Juel et al [214] speculate that it is likely to result from local, contraction-induced factors in the muscle, such as lactate production during training.

3.2.2.6 MCT's and hypoxia

Given that acute and chronic hypoxia affect lactate kinetics with exercise, it is possible that these changes are related to alterations in lactate transport and therefore MCT content. The effect of hypoxia on MCT expression is, however, still not entirely clear. While McClelland and Brooks [296] found that acclimation to chronic hypoxia had a muscle type specific effect on MCT1 and MCT4 expression in rats, Juel [216] and Clark [80] reported no change in either muscle MCT1 or MCT4 content after acclimation to altitude and 'live high, train low' hypoxic exposure, respectively. Certainly, the effects of chronic hypoxia on skeletal muscle MCT expression do not seem to be as marked as the effects of training.

3.2.2.7 MCT's and lactate-associated pathologies

It is also possible that pathologies that display abnormalities in lactate flux could result from aberrations in MCT expression. In 1986, Fishbein [139] described a muscular pathology involving reduced removal of muscle lactate after exercise and suggested that this could be the result of a deficient lactate transporter. More recently, Merezhinskaya et al [307] reported mutations in the MCT1 gene in patients with a deficiency in erythrocyte lactate transport and associated evidence of muscle injury after exercise with heat exposure. Regulation of MCT isoforms may therefore prove to be important for health and disease [179].

3.2.2.8 MCT's and endurance performance

Monocarboxylate transporters are important in endurance performance for multiple reasons. During physical activity, lactate is produced in skeletal muscle (especially in type 2B fibres) during glycolysis and glycogenolysis. The lactate levels in the cytosol must be prevented from rising to too great an extent for a high rate of flux in these pathways to be maintained, as is necessary during exercise. Lactate exits the cytosol either by entering the mitochondria or by being transported across the sarcolemma out of the cell. As such, the net lactate efflux from a muscle cell during exercise results from the difference between the lactate produced during glycolysis and the lactate taken up by the mitochondria. By transporting lactate out of the cell, MCT's are important in minimising lactate accumulation in myocytes with exercise [4].

In addition to transporting lactate across membranes, MCT's transport hydrogen ions, with one H^+ ion transported with each molecule of lactate in the same direction. As lactate and H^+ are transported across the sarcolemmal membrane together, lactate transport out of the cell is also of importance in muscle pH regulation. H^+ ions are produced together with lactate during glycolysis, which decreases cellular pH [80,243]. Regulation of intracellular pH is critical for cellular metabolism. Increased intracellular hydrogen ion concentrations can hasten fatigue through several mechanisms, such as decreasing the rate of ADP rephosphorylation, decreasing the cross-bridge cycle rate and increasing the rate of calcium reuptake [142,382]. Cellular pH homeostasis in muscle is a balance between H^+ accumulation and H^+ removal, mediated by the sarcolemmal transporters [210]. MCT's are not the only H^+ carriers out of the cell [17]. This task is also accomplished by the proton pump, the sodium-proton exchanger family, and the

bicarbonate transporter family [205]. MCT's play a fairly major role, however, such that the quantity of lactate released from muscle to blood is approximately two-thirds of total H^+ release during intense exercise in humans [211]. A high sarcolemmal density of MCT1 and MCT4 could therefore lessen perturbations in intracellular pH with exercise [4,376,377,400], thereby delaying fatigue.

According to the cell-cell and intracellular lactate shuttle models, lactate can be taken up by skeletal muscle and used as an oxidative fuel, especially in type 1 muscle fibres, which have a high mitochondrial density [59]. The transportation of lactate from the cytosol into the mitochondria is facilitated by the lactate concentration gradient between the cellular compartments, and because H^+ concentration is low and NAD^+ concentration high in the mitochondrial matrix from activity of the electron transport chain [63]. By facilitating the transport of lactate into muscle cells and into mitochondria, MCT's increase the availability of lactate as a respiratory fuel, aiding energy replacement during exercise. Monocarboxylate transporters are therefore of physiological importance to the maintenance of metabolic homeostasis during exercise [119], and are hence relevant to endurance performance.

3.2.2.9 MCT's and ethnicity

As described previously, different ethnic groups exhibit different athletic abilities in various forms of sport or physical activity. Differences have also been found between ethnic populations for blood lactate levels with exercise, as demonstrated in the previous chapter (Chapter 2). As MCT's are one of the factors regulating lactate in the body with exercise, they may play a role in these observed differences. Black South African distance runners, who generally outperform their white counterparts, have been found to have lower blood or plasma lactate concentrations during exercise than the white runners when compared at the elite [84] or the subelite [47,448] level. Coetzer et al [84] suggested that while the lower blood lactate concentrations in black runners could be the result of a difference in the rate of lactate accumulation, it could also reflect a difference in the rate of lactate removal from the blood or, indeed, the rate of lactate transport from the muscle into the blood. If there were a difference in the rate of lactate transport into or out of muscle cells, this could involve MCT variations between different ethnic groups.

Kenyan distance runners are also known to perform well in international events, and Saltin et al [388] found them to have lower blood lactate concentrations than Scandinavian runners at the same given exercise intensity. Saltin et al [387] found that the ratio of LDH isoform 1-2 to isoform 4-5 was higher in the Kenyans than the Scandinavians, suggesting that LDH may be related to the difference in blood lactate between the ethnic groups. However, the LDH ratios became much more similar between the groups after the Scandinavians had trained at altitude, suggesting that this difference may have environmental rather than genetic origins. Ama et al [8] have also reported ethnic variations in LDH, finding significantly higher muscle LDH activities in sedentary black subjects of West and Central African origin than sedentary white French Canadian subjects. MCT's and LDH work in conjunction to regulate the transport and oxidation of muscle lactate. It is therefore possible that differences in the skeletal muscle expression of one or more of the MCT isoforms could exist between people, including athletes, from different ethnic populations, although this has never yet been compared in different ethnic groups.

3.2.3 Summary

The metabolism of fuels to produce energy for muscle contraction increases with exercise. With the fatigue that occurs during prolonged exercise, there are changes in the concentrations of metabolites that are associated with muscle contraction, and these changes can affect the fatigue process either directly within the muscle or via afferents to the spine and brain. This affects the muscular contractile processes such that muscle fatigue is accompanied by a decrease in maximal muscle tension or force output, as well as a reduced power and shortening velocity. Human muscles are composed of a mixture of fibre types. Type 1 fibres are slow, oxidative fibres; type 2A are fast, oxidative-glycolytic fibres; and type 2B are fast, glycolytic fibres. Muscle fibre composition can affect athletic performance. While a high proportion of type 2 muscle fibres is advantageous for strength and power activities, a greater proportion of type 1 fibres is generally considered advantageous for endurance activities. The extent of fatigue during endurance activities may therefore be related to fibre composition, such that individuals with a high proportion of type 2 muscle fibres are more susceptible to fatigue than those with a high proportion of type 1 fibres. Muscle fibre composition is therefore often considered one of the main factors involved in determining endurance capacity. The

proportions of the muscle fibre types may also be different in different ethnic populations. One of the metabolic intermediates produced during muscular activity is lactate, which may represent an important means of distributing carbohydrate potential energy for oxidation and gluconeogenesis. Lactate is involved in metabolic communication between tissues, and functions as a substrate in energy metabolism in the heart, liver, skeletal muscle and brain. Monocarboxylate transporters facilitate the transport of lactate across muscle (and other) cell membranes, and are therefore important in the regulation of lactate turnover in skeletal muscle, and indeed the regulation of whole body lactate metabolism. MCT's also have a principal function in the provision of substrates for mitochondrial oxidation, and help to maintain redox balance in the cellular compartments. MCT1 and MCT4 are the two major MCT isoforms expressed in human skeletal muscle, with MCT1 thought to act predominantly as a lactate influx transporter and MCT4 predominantly as a lactate efflux transporter. A greater sarcolemmal (and mitochondrial) MCT content could delay the development of fatigue by reducing lactate and H^+ accumulation in the muscle cell, thereby lessening perturbations in intracellular pH, and by increasing the availability of lactate as a respiratory fuel in the mitochondria. Monocarboxylate transporters are therefore of physiological importance to the maintenance of metabolic homeostasis during exercise, and are hence relevant to endurance performance. In addition, it is possible that variations in muscle MCT concentrations between ethnic groups may play a role in the blood lactate concentration differences observed between ethnic populations with exercise.

3.3 INTRODUCTION

During prolonged exercise, fatigue is evident in the muscle as a reduced contractile ability, associated with decreases in muscle tension, power and shortening velocity [142]. This is often accompanied by changes in the concentrations of metabolites that are associated with muscle contraction [290]. Alterations in the levels of these metabolites can positively or negatively affect the fatigue process. One such metabolite is lactate, which is thought to be involved in whole body metabolism and metabolic communication between tissues [179], as well as functioning as a metabolic intermediate that presents a means of distributing carbohydrate potential energy for oxidation and gluconeogenesis [58,59,415]. Lactate levels in the muscle and blood increase with fatigue during exercise, and plasma lactate levels during exercise are related to endurance performance [416].

As described earlier, marked differences in plasma lactate levels with exercise were found between black and white South African runners in Chapter 2 of this thesis. This finding confirms previous reports of discrepancies in exercising lactate concentrations in South African runners from different ethnic groups that tend to perform to different standards in distance running competitions [47,84,448]. In addition, Kenyan distance runners, who are known to perform well in international events, have been found to have lower exercising blood lactate concentrations than Scandinavian runners [388]. This recurrent finding of low blood lactate levels with exercise in black African runners may be physiologically relevant to fatigue resistance during endurance performance and warrants further investigation.

One of the most important factors determining skeletal muscle lactate production, uptake and consumption is muscle fibre type [169,416]. Individuals with a higher proportion of type 2B fibres are likely to produce higher levels of lactate with exercise than individuals with a high proportion of type 1 fibres [223], which are metabolically suited for lactate oxidation [169]. Distance runners tend to have either equal numbers of type 1 and type 2 fibres or a predominance of type 1 fibres [338], and individuals with a high proportion of type 1 fibres are generally less susceptible to fatigue than those with a high proportion of type 2 fibres [434]. Fibre type composition has been investigated in black and white South African runners in two previous studies [84,448], both of which found no difference in fibre type proportions between the two groups. However, the sample size for fibre type

comparisons in these studies were low and it has been suggested that the published data is not sufficient to draw any definite conclusions as to whether or not there are any real differences between endurance athletes in African populations [329]. One of the aims of this chapter is therefore to confirm the reported fibre composition data with greater subject numbers.

The lactate transport capacity across the muscle sarcolemma is dependent on fibre type [169], and it is likely that most differences in lactate transport between muscle types are caused by a difference in the number of membrane transporter molecules [218]. As described in the literature review, recent work in the field of lactate metabolism in muscle has elaborated on the important role of monocarboxylate transporters (MCT's) in the transport and metabolism of lactate in exercising skeletal muscle. Therefore, as MCT's are one of the factors regulating lactate in the body, they may be related to the observed differences in blood lactate levels between different ethnic groups during exercise. Indeed, it has been previously suggested that lactate transporter density may be related to the difference in plasma lactate levels observed between South African black and white runners [448]. MCT isoform densities have, however, never yet been compared in different ethnic groups. This chapter will therefore examine MCT concentrations in black and white South African runners. In addition, training studies suggest that a greater skeletal muscle MCT content may be related to superior athletic capability [113], as a greater MCT content may delay the development of muscular acidosis and enhance performance. The relationship between the concentration of skeletal muscle MCT's and endurance performance is, however, not yet well understood [32]. The muscular concentrations of MCT1 and MCT4, the two major MCT isoforms expressed in skeletal muscle [212], will therefore be examined and related to running performance in this chapter.

3.4 METHODS

3.4.1 Subject characteristics

The subject characteristics were previously described in section 2.4.1 of Chapter 2. All 32 subjects were initially analysed together as a single group (Part A), and subsequently analysed as a group of 16 black runners and a group of 16 white controls (Part B). The reason for dividing the groups in this manner is described in the subject selection section of the thesis introduction (section 1.3).

3.4.2 Experimental design

The details of the experimental design were previously described in section 2.4.2 of Chapter 2. The design is also outlined in Figure 2.1 of Chapter 2. During the subjects' third visit to the laboratory they performed an interval run (as described in Chapter 2, section 2.4.4.4), after which they were allowed to rest for 20 min before a muscle biopsy was performed (section 3.4.3).

3.4.3 Muscle biopsy

Muscle biopsies were performed on subjects for the analysis of fibre type composition and muscle MCT1 and MCT4 content. Biopsies were collected from 22 subjects (12 black and 10 white subjects). Fibre type data was obtained from all 22 of these, however the data from four of the subjects was lost due to human error, resulting in final fibre type results from 8 black and 10 white subjects. MCT data was obtained from 19 of the 22 biopsy samples (12 black and 7 white subjects). The biopsy procedure was performed by a medical doctor. Subjects had the procedure fully explained to them prior to it being performed, with assistance from an interpreter when English was not the subjects' first language. The muscle biopsy samples (mean: ± 147 mg) were obtained from the belly of the vastus lateralis muscle of the left leg using the percutaneous needle biopsy technique of Bergstrom [24], as modified by Evans et al [125].

Briefly, the part of the thigh to be biopsied was injected with a local anaesthetic and cleaned with antiseptic. When the anaesthetic had taken effect, an incision was made through the skin and superficial fascia with a blade, allowing access to the muscle with a 5 mm biopsy needle. The needle was inserted into the muscle and the internal cutting cylinder retracted slightly. Suction was applied to the needle via an extension tube

attached to a syringe. The cylinder was closed and removed from the muscle. The incision was sealed with plastic stitches and pressure applied to the area with strapping. A portion of the muscle sample was separated from the rest for fibre type analysis. This was mounted on a piece of cork with embedding medium (Tissue-Tek, Miles Laboratories Inc. Naperville Illinois, USA), then frozen in liquid nitrogen-cooled isopentane and stored at -20°C for histological fibre type analysis (section 3.4.4.1). The remainder of the muscle sample was immersed in liquid nitrogen immediately after being obtained and stored at -80°C for subsequent MCT analysis (section 3.4.5). Some of the fibre type analyses were performed using electrophoretic methods, as described below (section 3.4.4 and 3.4.4.2), and in these cases the muscle used was part of the muscle that was immersed in liquid nitrogen and stored at -80°C.

3.4.4 Fibre typing

The fibre type compositions of the muscle sections were determined using one of two methods, namely histological myosin ATPase activity staining (section 3.4.4.1) and SDS-Polyacrylamide gel electrophoresis of myosin heavy chain isoforms (section 3.4.4.2). Two methods were used because the mounted muscle sections for histological fibre typing were lost for eight of the subjects due to freezer malfunction, and therefore the fibre compositions for these subjects were determined electrophoretically using separately stored muscle. In the results and discussion sections of this chapter (sections 3.5 and 3.6), the nomenclature used for the fibre types was type 1, type 2A and type 2B, with type 2B equating to the type 2X nomenclature that is sometimes used when fibre type is determined electrophoratically.

3.4.4.1 Histological Myosin ATPase staining

Myosin ATPase activity was determined by staining at pH 9.4, pH 4.6 and pH 4.3 [57]. Briefly, cryostat sections were cut (10 μm for pH 9.4 ATPase; 20 μm for pH 4.3 and pH 4.6 ATPase) and air-dried for at least 10 min. The sections were pre-incubated in the appropriate buffers (pH 9.4; pH 4.3; pH 4.6; Appendix C) for 15 min, after which all sections were incubated at pH 9.4 for 5 min. All sections were then incubated in a pre-warmed ATP solution (Appendix C) at 37°C for the appropriate times (pH 9.4: 7 min; pH 4.6: 17 min; pH 4.3: 32 min). The sections were washed in 90 mM calcium chloride and placed in 2 % cobalt chloride for 3 min (pH 9.4) or 6 min (pH 4.3 and 4.6). The sections were then washed in 10 mM sodium barbitone, rinsed in distilled water and the stain

colour developed in either 1 % or 7 % ammonium sulphide in a fume cupboard (1 % for pH 9.4; 7 % for pH 4.6 and pH 4.3). The slides were then washed in running water for 10 min to remove excess ammonium sulphide, dehydrated in alcohol, mounted in synthetic resin and covered with coverslips. The slides were examined using a light microscope and images of the stained sections captured with an interactive graphic digitiser (Carl Zeiss 'Axioplan' 2 MOT). Approximately 10 to 15 images were captured and approximately 400 muscle fibres counted per subject. Based on the myosin ATPase activity, the muscle fibres were classified as type 1, type 2A or type 2B, in accordance with the nomenclature of Brooke and Kaiser [57]. The fibre types were expressed as a percentage of the total number of muscle fibres counted.

3.4.4.2 SDS-Polyacrylamide gel electrophoresis of myosin heavy chains

In order to extract the myosin heavy chain proteins, each muscle sample was minced separately with scissors in Solution A (Appendix D), then centrifuged at 1614 × g for 7 min at 4°C, and the supernatant discarded. The protein pellets were resuspended in 3 volumes (of the muscle wet weight) extraction buffer. The samples were agitated at 4°C for 30 min, after which they were centrifuged at 1614 × g for 15 min. The supernatant was diluted with one volume of 87 % glycerol and stored at -20°C until further analysis. The myosin heavy chain proteins were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (Appendix D) for 24 hrs at 120 V, as described by Talmadge and Roy [426]. The gels were stained with Coomasie Blue stain, destained, photographed and scanned, and the relative density of each myosin heavy chain isoform quantified using a densitometer (Biorad, Hercules, CA, USA). The relative isoform content was expressed as the percentage each isoform contributed to the total density of the myosin heavy chain bands in each lane.

3.4.5 Monocarboxylate transporter analysis

MCT analysis was performed using a modification of the methods described by McCullagh et al [298] and Dubouchaud et al [113].

3.4.5.1 Protein extraction

Each muscle sample (mean size: ± 127 mg) was homogenised with a manual glass homogeniser at 4°C in 3 ml Buffer A (Appendix E) containing 4.5 µl protease inhibitor cocktail (Sigma, St Louis, USA). The muscle homogenate was transferred into a 15 ml

polypropylene tube and the homogenising tube rinsed with 1 ml Buffer A to make a total volume of 4 ml in the 15 ml polypropylene tube. The homogenate was centrifuged using a desktop centrifuge (Mistral 2000R, MSE, UK; swing bucket rotor) at 600 × g for 10 min at 4°C to eliminate red blood cell material. The resulting supernatant was transferred to a new centrifuge tube (10.4 ml polycarbonate, Beckman, CA, USA) with 0.75 vol of Buffer B and centrifuged (Beckman Optima™ L-70 Ultracentrifuge; 40Ti fixed angle rotor) at 145,000 × g for 2 hrs at 4°C. The resulting pellet was washed with 1 ml Buffer C and then resuspended in 200 µl Buffer C by passing the mixture through a pipette followed by a needle (21 gauge needle, 1 ml syringe). Sixty six µl of 16 % SDS was then added to the suspension, after which it was vortexed and centrifuged (Beckman J2-21; JA14 rotor) at 1100 × g for 20 min at 20°C. The supernatant was collected, divided into aliquots (± 30 µl) and stored at -80°C for subsequent Western blotting. Protein concentrations were determined spectrophotometrically (Beckman DU®-62) using the bicinchoninic acid assay according to the manufacturer's instructions (Pierce, Illinois, USA) with bovine serum albumin as a standard.

3.4.5.2 Western blotting

The compositions of the buffers used in the Western Blotting procedure are detailed in Appendix F. Five µg of each subject's protein was combined with sample buffer in a total volume of 20 µl, boiled for 5 min and loaded onto a 10 % polyacrylamide gel along with a molecular weight marker (Bio-Rad Broad Range, 161-0317). The proteins were separated by SDS-polyacrylamide gel electrophoresis at 150 V. A spare muscle protein sample was loaded and run in an identical manner on every gel to create an arbitrary unit to allow comparison of protein band densities between gels. The proteins were then transferred by electroblotting onto polyvinylidene difluoride (PVDF) membranes at 100 V for 1 hr. After transfer, the gels were stained with Coomassie Blue to confirm that transfer was complete. Additional gels were run, the proteins not transferred, and the gels stained with Coomassie Blue to confirm that equal concentrations of protein were effectively loaded onto the gel (Figure 3.1).

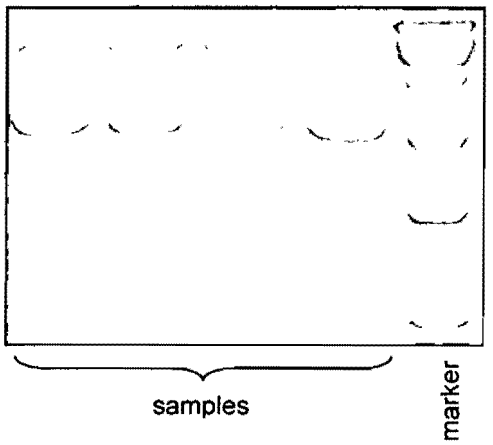


Figure 3.1: Representative gel of muscle protein samples, stained with Coomassie Blue, indicating protein bands.

The PVDF membranes were stained with 1 % Ponceau S to confirm that the proteins had transferred onto the membrane and to allow determination of the molecular weight marker bands and. The PVDF membranes were then destained in water and incubated overnight at 4°C in 20 ml of 10 % blocking buffer (Appendix F). The membranes were then incubated with anti-MCT1 antibody (0.142 μg/ml) for 2½ hrs at room temperature in 10 ml 5 % blocking buffer. The anti-MCT1 and anti-MCT4 antibodies were donated (G. Brooks, see Dubouchaud [113]). They were affinity purified polyclonal antibodies produced by immunising rabbits with synthetic peptides (as described by Poole, 1996), with the peptides corresponding to amino acids 483-500 of human MCT1 (SPDQKDTEGGPKEEESPV) and 440-455 of human MCT4 (LREVEHFLKAEPEKNG; Price, 1998). The membranes were washed in ± 50 ml TTBS (for 15 min and 2 x 5 min, Appendix F) and then in ± 50 ml 1xTBS (for 10 min, Appendix F). The PVDF membranes were then incubated in anti-rabbit secondary antibody (0.2 μg/ml affinity purified goat anti-rabbit immunoglobulin peroxidase labeled antibody, KPL, Maryland, USA), for 1½ hrs at room temperature in 10 ml 5 % blocking buffer, after which they were washed again as described above. MCT1 expression was detected using enhanced chemiluminescence as per the manufacturer's instructions (KPL LumiGLO®, Maryland, USA). Autoradiography films (Kodak Biomax ML, New York, USA) were exposed to the membranes and developed by hand (Sigma processing chemicals, Kodak GBX Developer and Fixer). The PVDF membranes were then incubated in ± 100 ml stripping

buffer (Appendix F) at 50°C for 30 min and washed as described above to remove the MCT1 antibody. The membranes were stained with 1 % Ponceau S to confirm that the proteins had not been removed with stripping, and then destained in water. The membranes were incubated overnight at 4°C in 20 ml 10 % blocking buffer and then with MCT4 (0.430 μg/ml) for 2½ hrs at room temperature in 10 ml 5 % blocking buffer. They were then washed as described above, incubated with anti-rabbit secondary antibody (0.333 μg/ml affinity purified goat anti-rabbit immunoglobulin peroxidase labeled antibody, KPL, Maryland, USA) for 1½ hrs at room temperature in 10 ml 5 % blocking buffer, and then washed again. MCT4 expression was detected and developed on film as described for MCT1. Films were photographed using a photodocumentation system (UVItec Ltd, Cambridge, UK) and band intensities were determined using UVIDocMw software (UVIDocMw version 99.04, UVISoft).

3.4.6 Statistical analysis

Statistical analyses were performed using the Statistica software package (Version 6, Statsoft, Tulsa, OK, USA). Correlations between physiological variables and performance variables (independent and dependent variables) in parts A and B were performed with the Pearson Product-Moment Correlation. In addition to comparing fibre type and MCT variables to performance, these two variables were also compared with each other, to investigate how fibre composition affects skeletal muscle MCT1 and MCT4 content. These correlations were also performed with the Pearson Product-Moment Correlation. When the data was divided into two groups based on ethnic origin (part B), comparisons of variables between the two groups were performed using the unpaired Students' t-test. Statistical significance was accepted when $p<0.05$. As described in section 3.4.3, fibre type and MCT data was not collected from all subjects, which is reflected in the subject numbers in the results.

3.5 RESULTS

3.5.1 Part A: Physiological variables and endurance performance

3.5.1.1 Fibre type

The fibre composition of the subjects' vastus lateralis muscle is shown in Table 3.1. The percentage of type 1 fibres was not significantly greater than the percentage of total type 2 fibres (type 2A + type 2B, 46.1 ± 16.8 %), although it was significantly greater than the percentage of type 2A and 2B fibres separately ($p<0.05$ and $p<0.001$, respectively (n=18)).

Table 3.1: Relative fibre type compositions of the vastus lateralis muscle for the subjects (n=18). Type 2B fibres equate to the type 2X fibres determined electrophoratically. Values expressed as mean ± standard deviation.

Fibre type	Fibre composition (%)	Range, min – max (%)
Type 1	53.5 ± 16.6	24.0 – 77.0
Type 2A	41.4 ± 15.7	21.0 – 76.0
Type 2B	4.7 ± 10.2	0.0 – 33.4

The percentage of type 1 fibres did not correlate significantly with 10 km personal best (PB) time (Figure 3.2). Similarly, there was also no significant correlation between 10 km PB time and total type 2 fibres, type 2A fibres or type 2B fibres (data not shown).

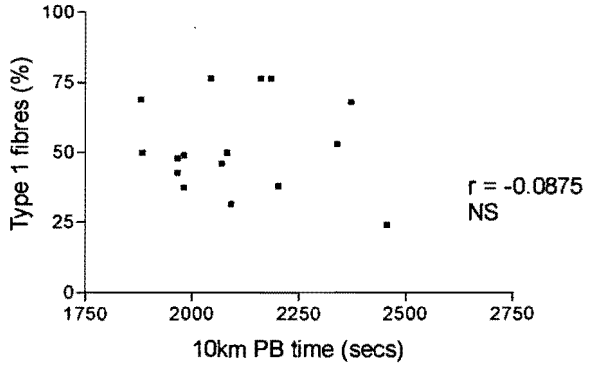


Figure 3.2: Correlation of percentage type 1 fibres in the vastus lateralis with 10 km personal best time (PB) for all subjects (n=16).

3.5.1.2 Monocarboxylate transporters

Representative Western blots indicating MCT1 and MCT4 content in muscle samples from the subjects' vastus lateralis are shown in Figure 3.3 and Figure 3.4, respectively. MCT1 and MCT4 expression was evident in all biopsies analysed.

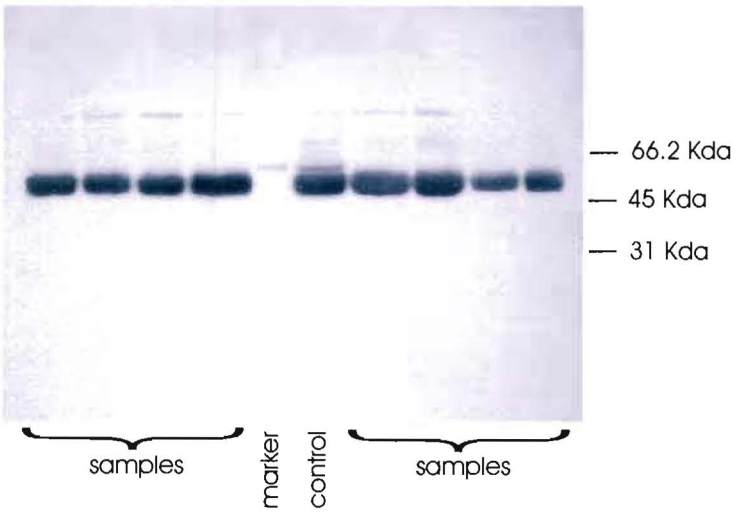


Figure 3.3: Representative Western blot indicating MCT1 content in muscle samples from the vastus lateralis. The sample lanes represent individual subjects. The control lane represents the control protein sample run on each gel to allow comparison of protein band densities between gels.

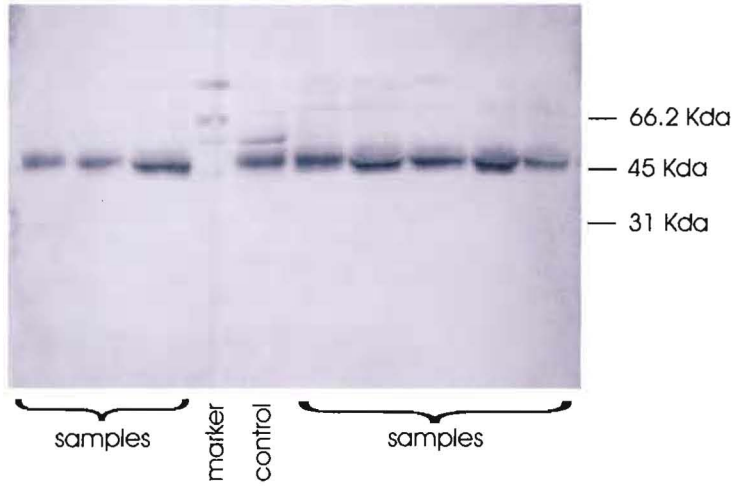


Figure 3.4: Representative Western blot indicating MCT4 content in muscle samples from the vastus lateralis. The sample lanes represent individual subjects. The control lane represents the control protein sample run on each gel to allow comparison of protein band densities between gels.

MCT1 content in the vastus lateralis did not correlate significantly with 10 km PB time (Figure 3.5).

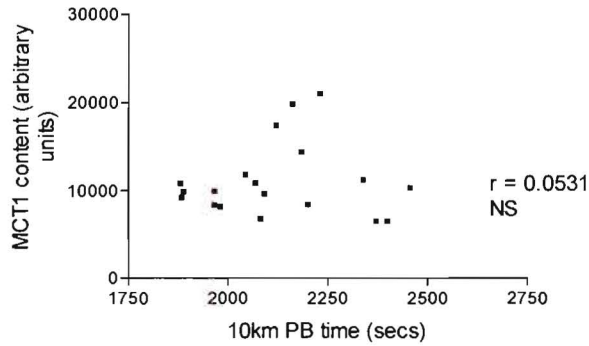


Figure 3.5: Correlation of MCT1 content with 10 km personal best time (PB) for all subjects (n=19).

MCT4 content, however, was significantly correlated with 10 km PB time ($p<0.05$; Figure 3.6).

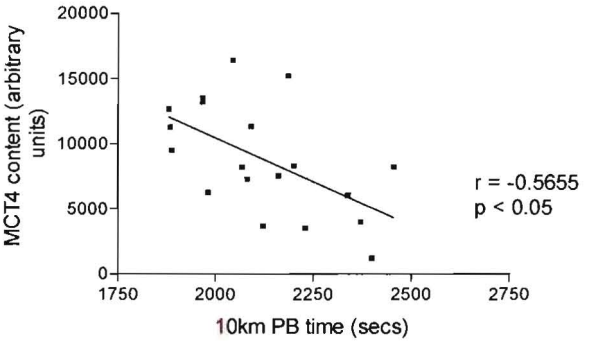


Figure 3.6: Correlation of MCT4 content with 10 km personal best time (PB) for all subjects (n=19).

The ratio of MCT1 to MCT4 content in the subjects' muscle did not correlate significantly with 10 km PB, although the correlation neared significance (Figure 3.7). There was also no significant correlation between the content of MCT1 and the content of MCT4 in the vastus lateralis (r=-0.078 and p=0.752).

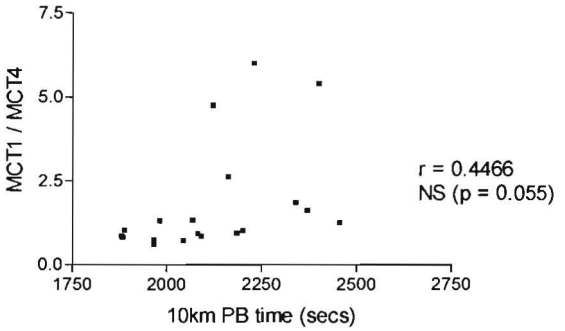


Figure 3.7: Correlation of the MCT1/MCT4 ratio with 10 km personal best time (PB) for all subjects (n=19).

3.5.1.3 Fibre type/MCT relationship

The MCT1 content of the vastus lateralis muscle did not correlate significantly with the percentage of type 1 fibres, although this correlation neared significance (Figure 3.8).

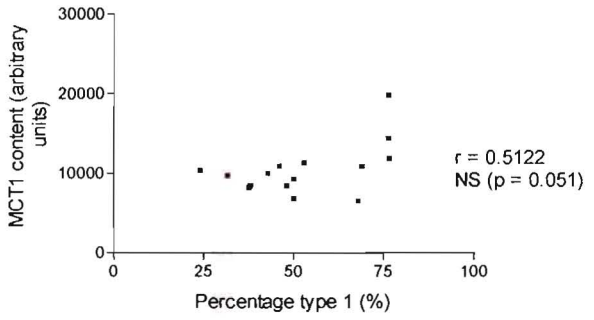


Figure 3.8: Correlation of MCT1 content with percentage type 1 fibres in the vastus lateralis for all subjects (n=15).

The MCT4 content of the vastus lateralis was not significantly correlated with the percentage of total type 2 fibres (Figure 3.9); nor did it correlate with either type 2A or type 2B fibres separately (r=-0.2127 and p=0.447, r=-0.0899 and p=0.750, respectively).

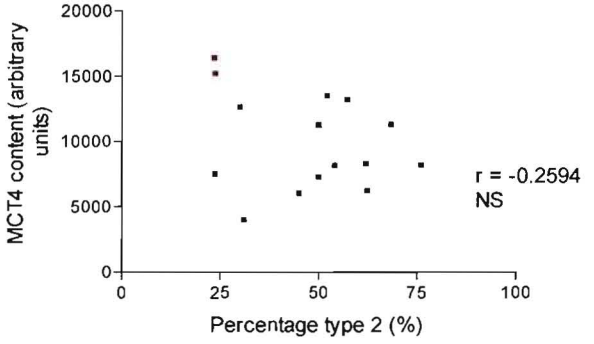


Figure 3.9: Correlation of MCT4 content with percentage type 2 fibres in the vastus lateralis for all subjects (n=15).

3.5.2 Part B: Ethnic comparison

3.5.2.1 Fibre type

There were no significant differences in the percentage of type 1, type 2A or type 2B fibres in the vastus lateralis muscle between the black and white runners (Table 3.1), or between the percentage of total type 2 fibres between the groups (black: 44.8 ± 18.6 %; white: 47.2 ± 16.1 %).

Table 3.1: Percentage of type 1, type 2A and type 2B fibres in the vastus lateralis of the black (n=8) and white (n=10) runners.

Fibre type	Black runners (%)	White runners (%)
Type 1	55.2 ± 18.6	52.1 ± 15.7
Type 2A	36.9 ± 13.7	45.0 ± 17.0
Type 2B	7.89 ± 14.6	2.20 ± 3.91

The percentage of type 1 fibres in the vastus lateralis did not correlate significantly with 10 km PB time for the black or the white runners (Figure 3.10). Similarly, 10 km PB time did not correlate with the percentage of total type 2 fibres, or the percentage of type 2A, or type 2B fibres in either group (data not shown).

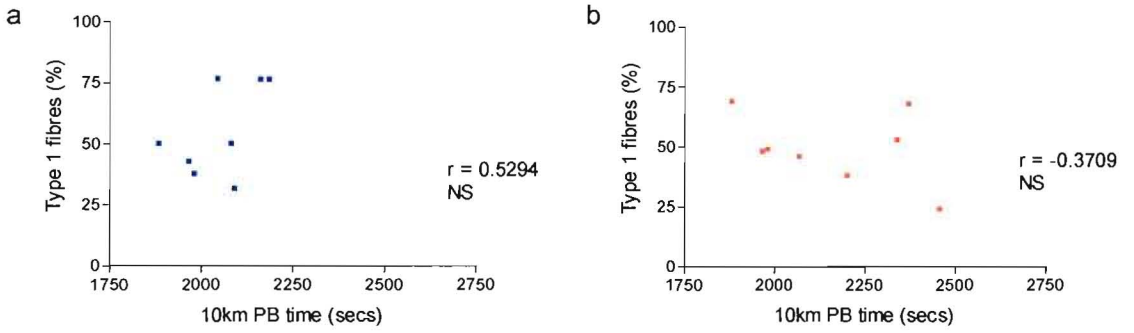


Figure 3.10: Correlation of percentage type 1 fibres in the vastus lateralis with 10 km personal best time (PB) for the black (a, n=8) and white (b, n=8) runners.

3.5.2.2 Monocarboxylate transporters

There was no significant difference in either MCT1 or MCT4 content in the vastus lateralis between the black and the white runners (Figure 3.11).

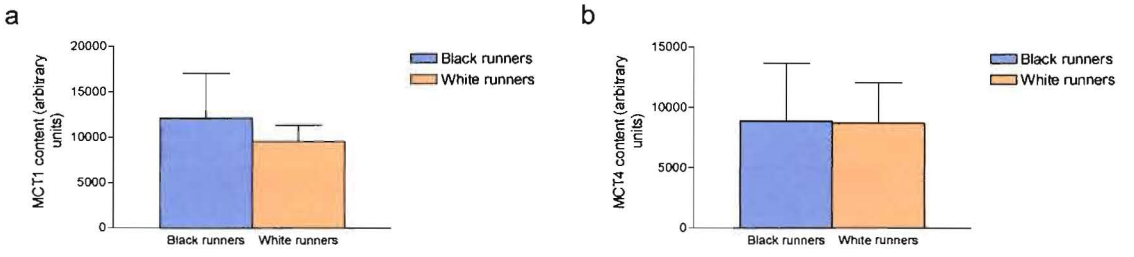


Figure 3.11: MCT1 (a) and MCT4 (b) content of the black (n=12) and white (n=7) runners.

MCT1 content did not correlate significantly with 10 km PB time for either the black or the white group (Figure 3.12). MCT4 content correlated significantly with 10 km PB time for the white runners, but not the black runners, although this correlation for the black runners neared significance (Figure 3.13).

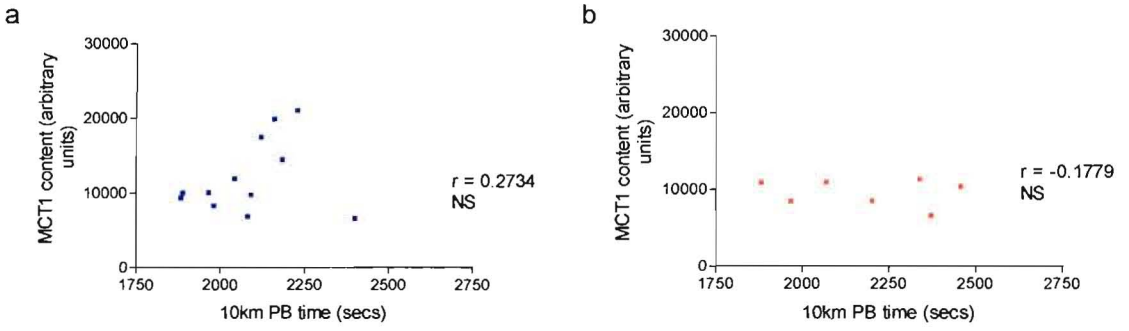


Figure 3.12: Correlation of MCT1 content with 10 km personal best time (PB) for the black (a, n=12) and white (b, n=7) runners.

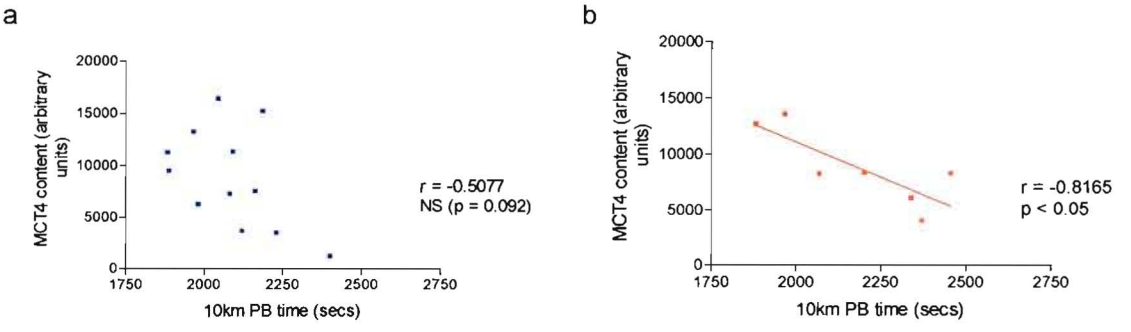


Figure 3.13: Correlation of MCT4 content with 10 km personal best time (PB) for the black (a, n=12) and white (b, n=7) runners.

The ratio of MCT1 to MCT4 content in the muscle was not significantly different between the black and white runners (Figure 3.14).

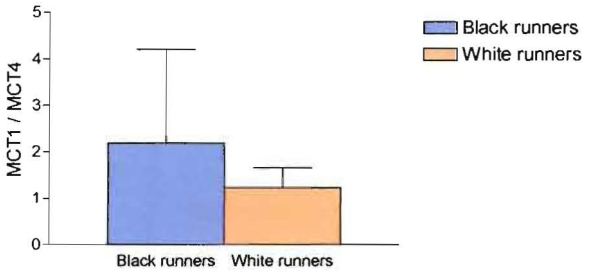


Figure 3.14: MCT1/MCT4 ratio for black (n=12) and white (n=7) runners.

While the ratio of MCT1 to MCT4 content correlated significantly with 10 km PB time for the black runners, it did not for the white runners, although this correlation for the white runners neared significance (Figure 3.15).

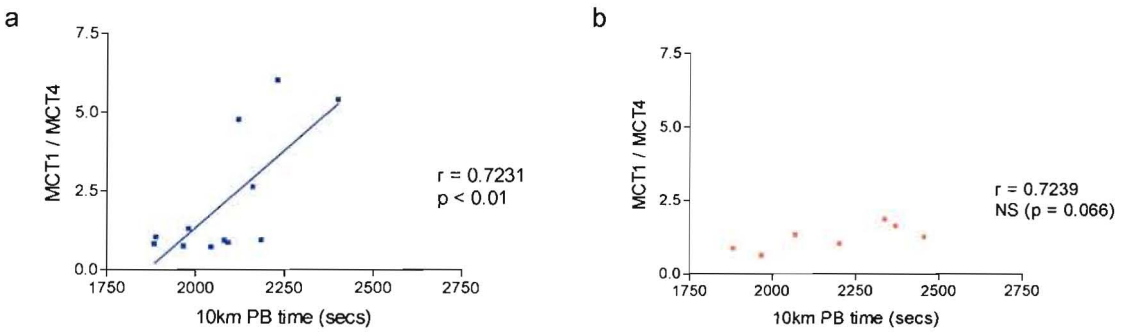


Figure 3.15: Correlation of the MCT1/MCT4 ratio with 10 km personal best time (PB) for the black (a, n=12) and white (b, n=7) runners.

3.5.2.3 Fibre type/MCT relationship

The MCT1 content of the vastus lateralis muscle correlated significantly with the percentage of type 1 fibres for the black runners (Figure 3.16 a), but not for the white runners (Figure 3.16 b).

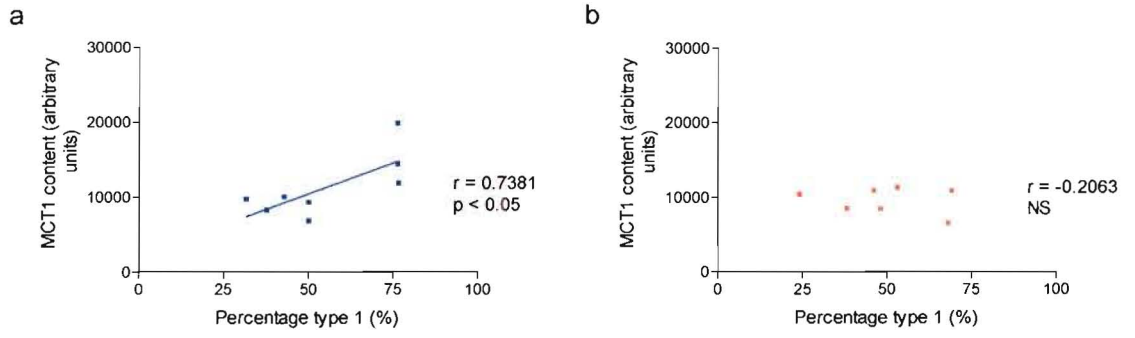


Figure 3.16: Correlation of MCT1 content with percentage type 1 fibres in the vastus lateralis for the black (a, n=8) and white (b, n=7) runners.

The MCT4 content in the vastus lateralis did not correlate significantly with the percentage of total type 2 fibres in either the black or the white group (Figure 3.17). There was also no significant correlation between MCT4 content and the percentage of type 2A or type 2B fibres separately, for either group (data not shown).

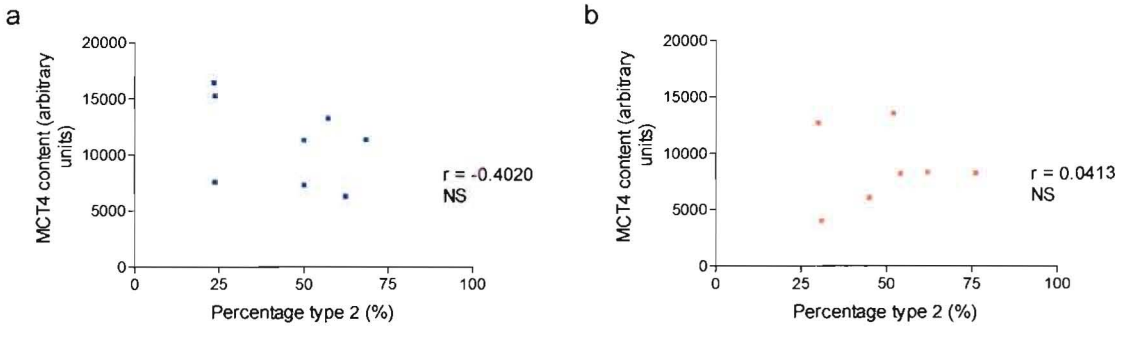


Figure 3.17: Correlation of MCT4 content with percentage type 2 fibres in the vastus lateralis for the black (a, n=8) and white (b, n=7) runners.

3.6 DISCUSSION

3.6.1 Monocarboxylate transporters

A novel finding of this chapter was that MCT4 content in the vastus lateralis was significantly correlated with 10 km personal best time. The correlation was negative, suggesting that the greater the MCT4 content in a well-trained runner's muscle, the better his performance in a 10 km race. When the two ethnic groups were analysed separately, MCT4 content correlated significantly with 10 km PB time for the white runners, and the relationship neared significance for the black runners. This again suggests that a larger muscle content of MCT4 is beneficial to endurance exercise, or at least to 10 km running performance. In addition, it suggests that the relationship between MCT4 content and performance is probably valid for both black and white (South African) athletes. The fact that the relationship was significant for the white runners and not the black may simply be the result of fairly modest sample sizes, as the curves for the two groups appear to follow a similar trend.

Skeletal muscle MCT's support the high rates of flux of lactate in the muscle with exercise [363]. Unlike MCT4, however, MCT1 content in the vastus lateralis did not correlate significantly with 10 km PB, either when the subjects were analysed together as one group, or separately as two ethnic groups. MCT4 and MCT1 are thought to have different roles in skeletal muscle [43], with MCT4 acting predominantly as a lactate efflux transporter [282,454], and MCT1 acting predominantly as a lactate influx transporter [73,364]. The increased amounts of lactate produced in the muscle with exercise need to be transported out of the cytosol in order to allow the continuation of glycolysis, so efficient removal of lactate from the muscle fibres might improve performance, especially during intense exercise [454]. MCT4 is suited for transporting lactate from the muscle under these conditions because it has a high capacity, is almost non-saturable and preferentially transports lactate over pyruvate under physiological conditions [110]. Therefore, by facilitating the removal of lactate from exercising muscle and therefore assisting the continuation of glycogenolysis and glycolysis, MCT4 may delay the development of fatigue during physical activity.

the differences in MCT1 content (standard deviations of 48.1 % and 37.7 %, respectively), confirming similar previous reports [113,361]. This larger interindividual variation in MCT4 may suggest that muscle MCT4 content is less tightly controlled than muscle MCT1 content.

The MCT1 and MCT4 content in the vastus lateralis were similar between the black and the white runners. This suggests that the total cellular MCT1 or MCT4 content in the muscle do not account for the observed superior performance of black compared to white South African runners. This finding also suggests that the total cellular MCT1 or MCT4 content in the muscle is unlikely to be responsible for the lower plasma lactate values observed in the black compared to the white South African runners with exercise (as found in the Cardiorespiratory factors chapter, Chapter 2). The MCT1/MCT4 ratio in the vastus lateralis was also similar between the groups, suggesting that the relationship between the transporters' muscle content is also not associated with performance or lactate differences between the ethnic groups.

This finding does not, however, preclude the involvement of MCT's in the observed ethnic discrepancy in exercising plasma lactate concentrations. As MCT's are found in subcellular fractions within the muscle cell [43,71], there could be a difference between the black and white athletes in the subcellular contents of MCT's. For example, MCT1 is found in the mitochondria, and is thought to facilitate lactate uptake into the mitochondria for oxidation [63,71,113]. If one ethnic group had a greater mitochondrial MCT1 content than another ethnic group they could theoretically have a metabolic advantage during exercise via enhanced cytosolic lactate clearance, more efficient fuel oxidation and greater cellular redox balance. Further investigation of the subcellular concentrations of skeletal muscle MCT's in black and white athletes is therefore recommended.

Variations in the efficiency of lactate transport across membranes may result not only from differences in MCT content, but also from differences in MCT activity [435]. It is therefore also possible that a difference in the regulation of the activity of skeletal muscle MCT's could be related to the differences in exercising plasma lactate levels in the black and white runners. In addition, muscle proteins other than MCT's could also be responsible for ethnic differences in lactate levels with exercise. For example, Saltin et al [387] found that the ratio of LDH isoform 1-2 to isoform 4-5 was higher in Kenyan runners

than Scandinavian runners, although the ratios became much more similar after the Scandinavians had trained at altitude. LDH catalyses the conversion between lactate and pyruvate in the muscle, and so could be associated with variations in muscle and plasma lactate levels with exercise. Comparison of the contents of the various LDH isoforms in the skeletal muscle of different ethnic groups therefore deserves further investigation.

It should be noted that the measurements of MCT content were only performed on the vastus lateralis muscle and extrapolation of the results to other muscles in the human body should be made with caution. In addition, the muscle biopsy from which the MCT data was measured was conducted approximately 20 minutes after the subjects had performed the interval running test. It is possible that this exercise bout may have affected the muscle content of the MCT's. For example, a decrease in sarcolemmal MCT4 content has been reported in rat muscle immediately after 10 minutes of intense contraction [435]. Whether the interval running test would have altered the resting muscle content of MCT1 or MCT4 in the runners tested in this thesis and, if so, whether this alteration would still be present after a short (20 min) period of rest is, however, unknown.

Analysis of the skeletal muscle MCT content in these runners therefore reveals that there appears to be an association between muscle MCT4 content and endurance performance.

3.6.2 Fibre type

Muscle fibre composition is often considered one of the main factors involved in determining endurance capacity [72], with many studies reporting that individuals with a high proportion of type 1 muscle fibres are less susceptible to fatigue during endurance activities than those with a high proportion of type 2 fibres [241,260,271,434]. It is interesting therefore, that there was no significant correlation between the percentage of type 1 fibres in the vastus lateralis and 10 km PB for the runners in this thesis. The percentage of type 1 fibres was also not significantly correlated with 10 km PB for the black or the white runners, when these ethnic groups were analysed separately.

The relationship between fibre type and endurance performance may be dependent on the nature of the endurance exercise and the training status of the subjects studied [272,292]. In this thesis, fibre composition was compared with a field-based measure of running performance, while many previous studies have compared fibre composition to the extent of fatigue during a laboratory-based endurance test (often only exercising a specific muscle group), with fatigue estimated by the decline in force output over time [241,260,271,434]. These laboratory-based fatigue tests may reflect fibre composition more closely than a field-based road race.

It has also been suggested that fibre composition is a better predictor of distance running ability when comparing athletes of differing ability, than when making a comparison within a group of athletes of a similar level [136]. Foster et al [146], for example, found that muscle fibre composition in runners from a broad range of abilities correlated with running performance over 6 miles (9.6 km). It is possible that the range of abilities of the runners in this thesis was not conducive to demonstrating a relationship between running performance and fibre composition. Nonetheless, the results from this thesis suggest that not having a high proportion of type 1 fibres is not necessarily detrimental to running performance. Indeed, type 2 fibres can be an important source of power generation during high running intensities, such as those during competitive 10 km races [448]. The importance of a high proportion of type 1 fibres in the skeletal muscle for distance runners, particularly those competing over shorter distances such as 10 km, is therefore arguable. It is possible that a high proportion of type 1 fibres in the skeletal muscle is more important during races of longer duration, such as marathons.

Previous research has shown that endurance-trained athletes often have a higher percentage of type 1 fibres in their skeletal muscle than untrained individuals or less trained athletes [136,204,257,260]. In this thesis, the percentage of type 1 fibres in the subjects' vastus lateralis muscle was slightly higher than that of the type 2 fibres, but this difference was not significant. This proportion of type 1 fibres (53.5 %) was either similar to or lower than that previously reported for endurance-trained athletes [146,204,251,260]. The greater proportion of type 2A fibres relative to type 2B fibres is also in agreement with previous research on endurance-trained men [204].

There were no significant differences in the percentage of type 1, type 2A, type 2B or total type 2 fibres in the vastus lateralis muscle between the black and white runners. This confirms the findings of the only other two studies to compare muscle fibre composition in black and white South African runners [84,448]. This lack of difference in fibre type proportions between the two groups suggests that fibre composition does not account for the observed superior performance of black compared to white South African runners. This finding also suggests that fibre composition is not the reason for the difference in exercising plasma lactate levels between black and white South African runners (as found in the Cardiorespiratory factors chapter, Chapter 2). Similarly, no difference in muscle fibre composition was found between black Kenyan and white Scandinavian runners [387], suggesting that fibre composition is not related to the reported difference in blood lactate levels between these ethnic groups either, or to the superior running performance of the Kenyan athletes.

In addition to the separate roles played by fibre type and MCT's during exercise, the fibre type of a muscle cell may influence its MCT content.

3.6.3 Fibre type/MCT relationship

While the MCT1 content of the vastus lateralis muscle did not correlate significantly with the percentage of type 1 fibres in this thesis, the correlation neared significance ($p=0.051$). The muscle MCT1 content did, however, correlate significantly with the percentage of type 1 fibres for the black runners, although this correlation was not significant for the white runners. Examined together, these results suggest that a relationship might exist between muscle type 1 fibre content and muscle MCT1 content, with a greater percentage of type 1 fibres being associated with a greater MCT1 content. This relationship would be in agreement with previous research [298,361], in which muscle MCT1 content correlated positively with the percentage of type 1 or oxidative fibres in skeletal muscle. This finding is consistent with the view that MCT1 is related to the oxidative nature of a muscle. Type 1 muscle fibres may have a greater MCT1 content than type 2 fibres in order to facilitate the uptake of lactate into these cells for oxidation within the mitochondria.

The MCT4 content of the vastus lateralis did not correlate significantly with the percentage of total type 2 fibres, either when all of the subjects were anlaysed together, or when the two ethnic groups were analysed separately. Similarly, MCT4 content did not correlate with type 2A fibres or with type 2B fibres, whether the subjects were analysed together or as separate ethnic groups. Muscle MCT4 content has been reported to be low in type 1 fibres and high in type 2 fibres [140,361,454], and has also been found to correlate positively with the percentage of type 2 glycolytic fibres in skeletal muscle [43]. Therefore, the findings of this thesis do not confirm these previous reports. Pilegaard et al [361] found that, while there was more MCT4 in type 2 compared to type 1 fibres when a muscle section was analysed using immunofluorescence microscopy, this difference was no longer evident when the fibre compositions of various muscles were correlated with the total MCT4 content determined by Western blotting. It is therefore possible that, while there was no relationship between fibre type and MCT4 content in the group of subjects as a whole, a relationship could exist between these two factors if they were compared in the muscles of subjects individually.

3.7 CONCLUSION

The findings of this chapter are summarised in Figure 3.17. The main finding of this chapter was that the MCT4 content in the vastus lateralis muscle of the runners was significantly correlated with their 10 km personal best time, such that the greater the MCT4 content, the better the race performance. This is suggested to be due to: 1) enhanced MCT4-mediated efflux of lactate from the muscle cell, allowing the continuation of glycolysis during exercise, and 2) the associated efficient efflux of H^+ ions, which would delay fatigue by preventing high levels of acidity in the cell. Muscle MCT1 content was not related to 10 km race performance. The MCT1/MCT4 ratio in the muscle correlated positively with 10 km personal best time for the black runners, and the relationship neared significance in the white runners and the group of runners as a whole. This is thought to be related to a favourable role for a large muscle MCT4 content in endurance performance. Neither MCT1 content, MCT4 content or the ratio of the two was significantly different between the black and the white runners. This suggests that the total cellular content of these MCT's in the muscle (or the relationship between their contents) is unlikely to be responsible for the observed difference in performance between black and white South African runners, or for the lower plasma lactate values observed in the black runners compared to the white runners during exercise. An ethnic comparison of the content of these MCT's in the mitochondrial and sarcolemmal fractions is recommended for future study. Unlike a number of previous reports, the percentage of type 1 fibres in the vastus lateralis did not correlate significantly with 10 km personal best time, perhaps due to the fairly restricted performance range of the runners in this thesis. There were also no significant differences in the percentage of type 1, type 2A or type 2B fibres between the black and white runners, or in the percentage of total type 2 fibres between the groups. This suggests that fibre composition does not account for the observed superior performance of black compared to white South African runners, or for the difference in exercising plasma lactate levels between the groups. The MCT1 content of the vastus lateralis muscle correlated positively with the percentage of type 1 fibres for the black runners, and the relationship neared significance for the subjects when analysed as one group. This is consistent with the view that MCT1 is related to the oxidative nature of a muscle. No relationship was found, however, between muscle MCT4 content and the percentage of type 2 fibres. In summary, therefore, neither the muscle content of MCT1 or MCT4, nor muscle fibre

composition can explain the observed performance difference between black and white South African runners. Muscle MCT4 content was associated with endurance performance, and further investigation of the role of MCT4 in fatigue and endurance performance is recommended.

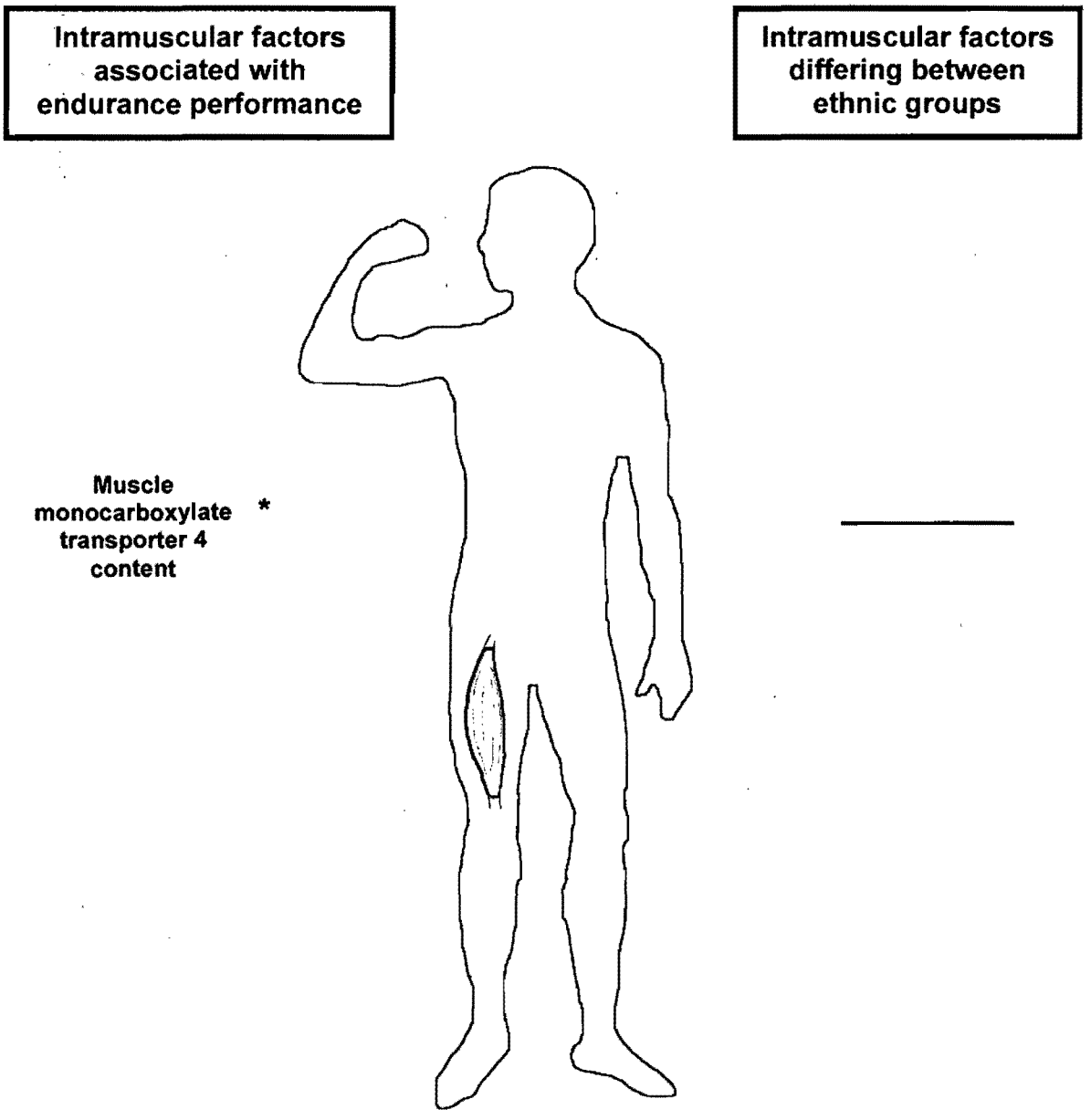


Figure 3.17: Summary of the intramuscular factors measured in this chapter that are associated with endurance performance and/or are different between black and white South African runners. * indicates novel finding

Chapter 4 Neuromuscular factors

4.1 PREAMBLE

In the Cardiorespiratory factors chapter (Chapter 2) it was found that running economy was correlated with endurance performance and was significantly better in the black South African runners than the white. This is probably related to the finding by Weston et al [448] that fatigue resistance during submaximal running was greater in black compared to white South African runners. Coetzer et al [84] similarly found that black South African runners exhibited a greater fatigue resistance than their white counterparts, but during a repeated isometric quadriceps contraction test. This suggests that there may be more to the black runners' fatigue resistance than simply advantageous running biomechanics or cardiorespiratory variables. This finding, however, may have been influenced by the black runners in the study being predominantly long distance runners while the white runners were predominantly middle distance track athletes. It is also not known if this fatigue resistance difference between the ethnic groups would be apparent during a sustained contraction. In addition, neuromuscular factors related to fatiguing activity have not been compared in black and white South African athletes.

Along with cardiorespiratory factors and factors related to muscle biochemistry, performance in distance running may be limited by factors related to the force and velocity characteristics of the neuromuscular system [335,350]. Neuromuscular factors may therefore also play a role in the performance difference between black and white runners. The differences found between the ethnic groups for plasma sodium and potassium may also be related to neuromuscular factors. For example the differences in plasma levels of these metabolites could be related to the functioning of the Na^+/K^+ pump, which is an electrogenic pump involved in muscle contraction and nerve conduction. These metabolites, along with lactate, are also involved in the stimulation of muscle afferents during exercise. Neural signals from the muscle and to the muscle can regulate contractile activity via changes in recruitment strategy, and the activity and properties of motor neurons can therefore be involved in minimising fatigue [157]. Endurance performance is affected by many components of the neuromuscular system, including both neural and muscular factors as well as the

use of elastic energy [349]. Indeed, elastic factors have also been suggested to be involved in the superior performance of black African runners [338].

This chapter will therefore investigate muscle endurance during static exercise, and the associated neuromuscular activity changes. Muscle elasticity will be investigated during stretch-shortening cycle performance and stride parameters will be examined.

4.2 LITERATURE REVIEW: The role of neuromuscular factors in fatigue and endurance performance

Performance in endurance activities, such as distance running, may be limited not only by cardiorespiratory factors (Chapter 2) or intramuscular factors (Chapter 3), but also by factors related to characteristics of the neuromuscular system [335,350]. Gandevia [157] has speculated that, if a muscle is regarded as a motor, then its behaviour is dependent not only on its intrinsic properties (Chapter 3), but also on the way it is driven, or controlled by the central nervous system, and the way feedback systems maintain its output. Motor neurons transmit electrical action potential signals that cause muscles to contract, while sensory receptors provide constant feedback via the nerves to the central nervous system, which modifies ongoing movements and initiates new movement [65]. A motor neuron and the muscle fibres it innervates comprise a motor unit [159], the functional unit of motor control in the body. The inherent excitability of these motor units is essential for movement and therefore physical activity [65].

The neuromuscular mechanisms involved in fatigue include all components of the motor system, from the production of motor drive in the central nervous system or changes in motor neuron excitability, to alterations at the neuromuscular junction, to alterations within the muscle fibres, including changes in sarcolemmal excitability, excitation-contraction coupling and contractile protein activity [122,446]. Therefore, peripheral sites in the muscle as well as central nervous sites are implicated in the fatigue process [157]. In addition to the neural control of muscle force production, the capability to store and utilise elastic energy is important in many forms of endurance activity, such as distance running [349].

4.2.1 Muscle strength and muscle fatigue

4.2.1.1 Motor unit recruitment and muscle force production

Muscle force production is affected by neural as well as muscle factors, and as such can be altered by changing the number of active motor units, the type of muscle fibres recruited and the motor unit firing rate [65,220,252]. Type 2 muscle fibres are capable of producing a greater force output than type 1 muscle fibres [50,51] and are therefore important in the production of large force outputs. The main factor that increases muscle force output at low force levels is motor unit recruitment, while rate coding (adjustment of motor unit firing frequency) is important at high force levels [323].

The average motor unit conduction velocity increases with the level of muscle force output, reflecting both changes within motor units and changes in the type of motor unit recruited [360]. Motor unit recruitment may also be affected by the availability of oxygen and blood-borne energy substrates [323]. The availability of these substrates as well as the removal of products of metabolism could therefore affect motor unit recruitment during fatiguing activity. Neural activation and the resulting muscular action are also different between different types of contraction [231], and maximal force output levels and neuromuscular fatigue profiles differ for isometric, concentric and eccentric muscle activity [228].

It has been proposed that the order in which motor neurons are recruited is related to their size [189]. Smaller motor neurons are more susceptible to discharge than larger neurons, with the probable basis for this being that there is a larger excitatory postsynaptic potential in the smaller cells than the larger ones [279]. Binder et al [37] found that this orderly recruitment did not merely occur as a result of there being different motor neuron types as recruitment order correlated with cell size when cells of a single histochemical type were examined. Furthermore, Enoka et al [120] found that recruitment, derecruitment and the discharge pattern of motor unit activity changed with fatigue, with the variation in recruitment order apparently increased compared to the non-fatigued state.

4.2.1.2 Central and peripheral fatigue

With prolonged exercise there are metabolic changes in the active motor units, which can lead to a decrease in the force-generating capacity of the muscle through many mechanisms [258]. As described earlier, these mechanisms involve all components of the motor system, from central drive through to muscle protein activity [122]. Fatigue is therefore often broken down into central fatigue, defined as "a progressive reduction in voluntary activation of muscle during exercise" and peripheral fatigue, namely "fatigue produced by changes at or distal to the neuromuscular junction" [157]. The relative contributions of peripheral and central fatigue during endurance activity, however, is still under investigation [227,275,341,395].

The decrease in voluntary activation with central fatigue may serve to protect the intracellular events accompanying excitation-contraction coupling from entering into a detrimental state from which recovery is retarded or even impossible [157]. Central fatigue may also serve to protect other essential homeostatic systems, such as the maintenance of temperature, blood pressure and ventilation [157]. Noakes [337] similarly

proposed that skeletal muscle recruitment is regulated by a central "governor" which acts to prevent a progressive myocardial ischemia that would precede skeletal muscle anaerobiosis during maximum exercise. Kayser et al [230] found that muscle recruitment was reduced at peak exercise in chronic hypoxia (instead of the typical increase which would indicate peripheral neuromuscular fatigue) and concluded that exhaustive dynamic exercise under these conditions may be limited by central nervous rather than peripheral metabolic fatigue.

4.2.1.3 Muscle activity and metabolic changes with fatigue

With fatigue during an isometric contraction of a limb muscle, there is often a progressive recruitment of other muscles. This is described as 'synkinesis' [157], and this additional recruitment of other muscles can help to maintain force output during fatiguing activity. Synkinesis can involve contraction of muscles ipsilateral and contralateral to the exercising limb during both maximal and submaximal exercise, and can occur prior to any decrease in force from the contracting muscles [109]. During a fatiguing exercise, muscle recruitment may also rotate between synergist muscles (intermuscular rotation), possibly in order to spare muscles from fatigue by using different biomechanical approaches to perform the task [188].

Intramuscular rotation among motor units (or motor unit substitution) may also occur during a fatiguing task, although this issue is still debated [157]. Zijdewind et al [459] performed surface and intramuscular EMG recordings during a fatiguing contraction of the first dorsal interosseous muscle and found that the recordings were markedly different between the two. They concluded that the responses of the muscle to fatigue, detected using EMG, have a heterogenous intramuscular distribution. Westgaard and De Luca [447] found that motor unit substitution occurred during long-duration submaximal isometric contraction of the trapezius muscle. They speculated that this substitution process would protect motor units from excessive fatigue. Olsen et al [347], however, examined motor unit firing patterns during a sustained static contraction of the extensor carpi radialis muscle and found that most motor units showed a continuous firing pattern after recruitment with no obvious incidences of rotation.

The muscle fibre membrane permeability appears to change with fatigue, affecting the normal propagation of action potentials and causing a decrease in the MFCV [220,233,381]. This change in conduction velocity may result from a decrease in ATP availability associated with increasing fatigue, as active metabolic processes are

required to maintain the Na^+/K^+ pump [220]. The decrease in MFCV during a fatiguing contraction may also reflect an accumulation of metabolic byproducts [56,264,290]. Masuda et al [290] found that MFCV decreased significantly during a fatiguing static contraction, but not during a fatiguing dynamic contraction. They concluded that this difference was a result of muscle blood flow being better maintained by enhanced venous return from the contracting muscle during the dynamic contraction compared to the static contraction. The difference in muscle blood flow would result in different metabolic states in these two types of contraction, which would in turn affect the MFCV. Changes in MFCV could also be caused, however, by changes in the motor unit recruitment pattern, such as by a shift in recruitment from type 2 to type 1 fibres [395]. Type 2 fibres generally produce action potentials with greater amplitude and faster depolarisation and repolarisation than type 1 fibres [442]. Type 2 fibres therefore have higher conduction velocities than type 1 fibres [220]. The fatigue profiles of the muscle fibre types may also differ, as discussed in the Intramuscular factors chapter (Chapter 3).

In this previous chapter (Chapter 3), it was argued that differences in skeletal muscle characteristics are one of the potential sources of ethnic differences in athletic performance. Similarly, the neural recruitment of muscle could be a distinguishing factor in the performance of different ethnic groups.

4.2.1.4 Muscle strength, muscle fatigue and ethnicity

In a study comparing sedentary white Canadians and sedentary black subjects of West and Central African descent, Ama et al [7] found no difference between the groups for maximal isometric force output or total work output during repetitive knee extension tests. They found, however, that the mean decrease in force generation during 90 seconds of repetitive knee extensions was greater in the black than the white subjects, suggesting that the black subjects were less resistant to fatigue than the white subjects were. In contrast, Coetzer et al [84] studied elite black and white South African middle- to long-distance runners and found that the time to fatigue during repetitive isometric muscle contractions was longer in the black than the white runners, suggesting that the black runners were more fatigue resistant during this type of exercise than the white runners were (Table 2.1, Chapter 2). Although on the surface this finding appears to contradict that of Ama et al [7], the results of these studies agree with the anecdotal evidence that black people of West African descent perform well in sprint rather than endurance events, while black people of South or East African descent perform well in endurance activities. Coetzer et al [84] also found

that the South African black runners had a lower peak isometric muscle strength than the white runners, when expressed as an absolute torque or when expressed per lean thigh volume.

Research of neuromuscular function often makes use of electromyography (EMG), which is used in many different types of studies, including those examining muscle endurance capacity and muscle fatigue [323].

4.2.2 Electromyography and muscle stimulation

The electromyogram is a composite of all the active motor unit action potentials being recorded, and can be affected by many characteristics, including technical factors like type of electrode use and signal processing, as well as physiological factors such as tissue filtering and muscle fibre characteristics [220]. Muscle fibre characteristics include the number of motor units recruited, their size, type and firing rate, and the degree of synchronisation of firing of the motor units [137]. It appears that differences in environmental and skin temperature, however, do not affect the EMG signal during exercise [200]. Many different techniques are employed to analyse the EMG signal and the merits and disadvantages of many of these methods are still debated by electrophysiologists.

4.2.2.1 Stationarity and normalisation

The issue of stationarity, for example, is still controversial. When analysing the EMG frequency spectrum it is ideal for the recorded signal to be stationary, in other words, the characteristics of the signal are relatively stable for the period of time recorded. The EMG signal is, however, non-stationary for dynamic contractions as well as sustained fatiguing contractions [129]. Most exercise physiology studies incorporating spectral analysis assume stationarity rather than actually testing for it [36]. EMG signals from sustained isometric contractions can be assumed to be stationary for short periods of time [36,129]. Bilodeau et al [36] also found that for short (512 ms), finite time windows, EMG signals have the same stationarity characteristics whether obtained from ramp or steady force level contractions, and over a range of force levels.

Another contentious issue in electrophysiology is normalisation of data. Normalisation of EMG data involves expressing the data relative to a reference value. While the importance of normalisation of EMG data is acknowledged by researchers, the methods for performing the normalisation are still controversial [69,198].

Normalisation methods that express the EMG data relative to the data obtained from a maximal voluntary contraction are the only ones that can indicate what percentage of maximum muscle recruitment is being used during a task [69]. Burden and Bartlett [68] compared four different EMG normalisation methods during contractions of the biceps brachii and concluded that normalising to an isometric or isokinetic MVC was preferable to normalising using the 'dynamic peak' or 'dynamic mean' method if the objective is to compare EMG data between muscles, tasks or individuals.

4.2.2.2 EMG amplitude and frequency

Analysis of the EMG signal to gain insight into muscle activity often involves the calculation of the amplitude and the frequency content of the power spectrum of the electromyogram. Variables obtained from the EMG signal are estimates, with values that depend on the calculation used to generate the estimated value and the window length sampled [129]. For example, EMG amplitude may be expressed by the root mean square value or the average rectified value, and the frequency content of the signal may be expressed as the mean spectral frequency or the median frequency [129]. These signal processing techniques are based on different assumptions and therefore have advantages and disadvantages in their use in describing EMG activity.

Surface EMG amplitude is dependent on both the recruitment and the firing rates of active motor units [130]. The EMG amplitude recorded from a muscle increases with increasing force output from that muscle, reflecting recruitment of new motor units, recruitment of larger motor units and increases in motor unit firing rate [137,166,224,242,320,423]. Suzuki et al [423] found that the change in mean surface EMG amplitude with force could be represented by the mean of the product of motor unit size (represented by motor unit action potential area) and mean firing rate. It should be noted, however, that with additional motor unit recruitment, it is more likely that motor units close to the recording electrode will be recruited. This will involve less tissue filtering of the EMG signal and therefore also affect the amplitude recording [137].

Reports on the change in EMG frequency content with increasing force output have been inconsistent. Moritani and Muro [320] and Karlsson and Gerdle [224] found an increase in EMG mean frequency with increasing force output, while Rainoldi et al [369] found a decrease. Reasons for the inconsistent findings for the relationship between force output and EMG frequency may include the methods used for frequency recording and analysis, thickness of the skinfold below the EMG electrode,

differences in muscle morphology (including fibre composition), gender differences, and the contraction type performed to obtain the frequency–force relationship [35,224].

The frequency content of the EMG signal is also related to the number and type of motor units active as well as their firing rates [220]. Moritani and Muro [320] found that the increases in surface EMG amplitude and mean power frequency with increasing force output were accompanied by increases in the firing frequency of motor units initially recruited, and of newly recruited motor units with relatively larger spike amplitudes. The recruitment of larger motor units with an increase in force output will result in an increase in the average conduction velocity (as this is proportional to fibre diameter), which in turn affects the frequency estimate [224].

4.2.2.3 Effects of fatigue on the EMG signal

The amplitude and the frequency content of an EMG signal are affected by both nervous and muscular factors and so reflect both peripheral and central features of the neuromuscular system [130]. The EMG signal therefore also reflects both peripheral and central fatigue processes. For example, alterations in metabolite concentrations in the muscle during fatiguing activity can affect muscle excitation-contraction coupling, including muscle membrane properties and action potential propagation, which could result in changes in the EMG signal [323]. Similarly, a change in the descending motor command from the central nervous system will alter muscle recruitment and hence affect the EMG signal. Fatigue also affects EMG amplitude and frequency differently depending on what type of contraction the muscle being recorded from is performing. For example, EMG amplitude and frequency content may differ between a static and a dynamic fatiguing contraction [290]. The EMG signal also differs during fatiguing isometric contractions depending on the percentage of maximal voluntary contraction maintained [137].

4.2.2.4 Effects of fatigue during a submaximal contraction on EMG amplitude

EMG amplitude increases with time during a fatiguing submaximal contraction in which force output is maintained. This increased amplitude occurs during both submaximal static and dynamic fatiguing exercise, for many different muscles and over a range of contraction intensities [151,273,328,423,439,446]. Masuda et al [290] have found that amplitude values were higher for dynamic compared to static fatiguing knee extensions.

There are many factors that could contribute to this increase in EMG amplitude during constant force output fatiguing activity. Perhaps the most commonly described reason for the observed change in EMG is the progressive recruitment of additional motor units in order to maintain a constant force output as already active motor units fatigue [220,323,423,446]. This augmented motor unit recruitment compensates for the decrease in contractile capability of the fatigued motor units [220]. The rate of increase of EMG amplitude during a fatiguing submaximal isometric contraction is greater the higher the level of force output, suggesting that additional motor unit recruitment might occur earlier and to a greater degree during contractions with a larger force output [423]. It is possible that more motor units with larger action potentials are recruited with time [423]. Furthermore, Moritani and Yoshitake [323] suggested that there could be a decrease in the recruitment threshold force of type 2a and 2b fibres with fatigue, such that more of these types of fibres are recruited as fatigue progresses. Other factors that have been suggested to be involved in increases in EMG amplitude during fatiguing activity are an increased motor unit firing frequency, increased motor unit synchronisation and changes in action potential propagation [423,446].

During submaximal contractions in a non-fatigued state not all of the available motor unit pool is recruited [323]. With the progression of fatigue during a submaximal contraction, however, this remains the case. EMG amplitude has been reported to be significantly less than maximum at the endurance limit of sustained submaximal isometric contractions [151,446]. St Clair Gibson et al [413] found that EMG amplitude values were less than 20% of maximal during a 100 km cycling time trial. However, as this trial did not require a constant force output and instead required a set distance to be completed, they found that EMG amplitude decreased, rather than increased, with time during the test. As the EMG amplitude values were substantially less than maximal and decreased with time, the authors concluded that central deactivation of muscle occurs during prolonged submaximal exercise in which force output can be varied.

4.2.2.5 Effects of fatigue during a submaximal contraction on EMG frequency

During a sustained submaximal muscle contraction, there is frequency compression (or a left shift) of the power spectrum of the EMG signal [135]. The mean or median frequency therefore decreases with time during a fatiguing submaximal contraction in which force output is maintained. This decrease is evident for many different muscles, during both static and dynamic contractions, and over a range of

contraction intensities [151,273,290,328,439]. The rate of decrease of the mean or median frequency with fatigue during a sustained submaximal contraction is dependent on the force of the contraction, with the rate of decrease greater for contractions of a higher force level [276]. The EMG power spectrum is related to the duration, amplitude and firing frequency of the motor unit action potentials [137]. Changes in more than one factor can therefore lead to the shift in the frequency spectrum with fatigue.

A shift in the EMG power spectrum to lower frequencies can be caused by an increase in the duration of motor unit action potentials, which is partly caused by a decrease in MFCV [290]. The change in EMG frequency with fatigue may therefore be the result of a decrease in MFCV [381]. However, EMG frequency has been found to decrease by a relatively greater amount than MFCV during fatiguing submaximal static exercise [276,290,369]. MFCV is therefore not the only factor causing the EMG frequency shift with fatigue [276,290]. The frequency content of the EMG signal can also be related to the number and type of active motor units, as well as their firing rates [220]. It has been suggested that changes in mean power frequency with fatigue are particularly sensitive to metabolic changes in type 2 fibres, and that the frequency shift with fatigue mainly reflects peripheral fatigue of the type 2 fibres [165,167].

Therefore, EMG amplitude increases and EMG frequency decreases during fatiguing submaximal contractions of constant force output, and there are many factors that may cause these changes in the EMG signal. One of the methods used, often in combination with EMG measurement, to investigate the contribution of peripheral and central factors in fatigue is muscle stimulation.

4.2.2.6 Examination of fatigue using muscle stimulation

Estimation of peripheral fatigue during or after a fatiguing contraction has been performed by observing the effects of electrical muscle stimulation on force output [158,232,395]. Peripheral fatigue is shown by the decrease in evoked twitch torque after a fatiguing contraction compared to the twitch torque prior to the contraction [151,446]. Muscle stimulation has also been used to demonstrate fatigue-induced changes in muscle contractility. Fatigue induced by a sustained maximal voluntary contraction results in an increase in stimulated twitch duration, with a decrease in the rates of muscle contraction and relaxation [30,395].

Electrical muscle stimulation has also been used to show that fatigue has a central component. Loscher et al [274] found that when the voluntary endurance limit during a

sustained submaximal contraction was reached, electrical stimulation of the muscle could generate the same torque level as the voluntary contraction for an additional minute in most subjects. The authors concluded that central fatigue had occurred during the contraction, as electrical stimulation could increase force output to the required level when voluntary contraction could not. Using a similar protocol, Loscher et al [275] again investigated central fatigue by superimposing evoked twitches on a sustained submaximal isometric contraction. EMG amplitude increased throughout the contraction, while superimposed twitch amplitude decreased, yet was not eliminated at the endurance limit. The authors concluded that, as the twitch was not eliminated at the voluntary endurance limit and the EMG did not reach its unfatigued maximal voluntary level, that central fatigue had occurred during the contraction. It appears, however, that different fatigue processes may be occurring during sustained maximal, rather than submaximal, contractions, as maximal tetanic stimulations failed to increase the voluntary contraction force output during a sustained maximal contraction [322].

4.2.2.7 Effects of fatigue during a maximal contraction on EMG amplitude and frequency

Similar to during a sustained submaximal contraction, the EMG power spectrum shifts to lower frequencies during a sustained maximal voluntary contraction [28,228,321,328]. Unlike during a sustained submaximal contraction, however, EMG amplitude decreases during a sustained maximal voluntary contraction [26,31,228,322,328]. Kay et al [228] found that EMG amplitude decreased along with force output during a 100 s isometric maximal voluntary contraction (MVC) and concluded that this decline in EMG amplitude was the result of a decrease in neural drive to the muscle, in other words, central fatigue. It was not evident, however, whether the decreased neural drive was initiated by a pre-programmed central nervous system activity or was a response to changes in afferent input resulting from metabolic alterations in the muscle.

This decrease in EMG amplitude during fatiguing maximal activity also appears to occur during dynamic exercise. Paavolainen et al [349] found a lower EMG amplitude during a 20 m sprint in a fatigued state, compared to that during a sprint performed in an unfatigued condition. They concluded that this reduced neural input to the muscles meant that the fatigue induced by the run might have been not only peripheral, but also central in origin. In contrast, Hunter et al [199] found that, despite a decline in mean power frequency, there was no change in EMG amplitude during a

'supramaximal' cycling protocol (30 s Wingate test). They suggested that this could either reflect an accumulation of metabolites in the periphery, or an inability of the feedback loop from intramuscular metabolism to the central nervous system to affect neural recruitment strategy within the 30 second period. It should briefly be noted at this point that muscle recruitment and force output during a fatiguing MVC, or in fact even during a short MVC, is not truly 'maximal' [157], hence the term 'voluntary'. These tests nonetheless represent the maximal effort that the subject is willing to produce under the given conditions, and hence are regarded as 'maximal'.

The reduction in EMG mean power frequency and EMG amplitude, and hence the reduction in motor unit activity, during sustained MVC's may be muscle fibre type dependent [322]. As with fatigue during sustained submaximal contractions, the changes in these EMG parameters with fatigue during sustained MVC's may result from a number of factors. As mentioned during the discussion of submaximal contractions, a decrease in MFCV has been suggested to cause the left shift in the EMG power spectrum [381] with fatigue during a maximal contraction. Bigland-Ritchie et al [28], however, found that the changes in conduction velocity with a fatiguing maximal contraction were not enough to account completely for the changes that occurred simultaneously in the frequency power spectrum. A decline in motor unit firing frequency accompanies the decreases in EMG frequency and EMG amplitude during a sustained MVC, and this could indirectly result from a decrease in the contractility of the muscles involved, which could be causing the reduction in the muscle force generating capacity [31,322]. This is because the motor neuron firing rate elicited by voluntary contraction may be regulated according to the contractile state of the muscle to be the minimum required for maximum force production, in order to prevent neuromuscular transmission failure and optimise motor control [31]. This concept is part of the theory of muscle wisdom.

4.2.2.8 Muscle wisdom

According to Gandevia [157], 'muscular (or muscle) wisdom' refers to the decline in motoneuron firing frequency that occurs during a sustained maximal voluntary contraction, with the firing rate of the motoneuron decreasing to match the altered contractile properties of the muscle. This firing pattern change theoretically provides an efficient maintenance of force output. This hypothesis is supported by evidence from various studies. Bigland-Ritchie et al [29] found a decline in motor unit firing rates with fatigue during a MVC, and suggested that this decrease in the motor unit discharge rates allowed modulation of voluntary force output by rate coding to allow

the subject to continue contracting during fatigue. In a second study, Bigland-Ritchie et al [30] measured the change in muscle contractile speed during a sustained MVC and found that there was a progressive slowing of contraction speed such that the excitation rate required for maximal force generation was reduced. Moritani et al [321] compared muscle fatigue during an MVC to that when the muscle was electrically stimulated to contract. They adjusted the stimulus voltage so that force generated by the stimulation initially matched the force of the MVC. They found that after 30 s of high-frequency stimulation, significantly less force was generated than after a similar period of MVC. The decrease in force during the high-frequency tetanic contractions was accompanied by a reduction in the evoked potential amplitude and conduction time. The authors therefore concluded that their data supported the hypothesis that fatigue during high-frequency stimulation results from failure of electrical propagation due to reduced muscle membrane excitability. Bigland-Ritchie [26] performed an investigation of the decrease in force output during an isometric MVC of the adductor pollicis muscle. Similarly to Moritani et al [321], they found that if they performed continuous nerve stimulation at a frequency that matched the voluntary force output of the unfatigued muscle, there was a progressive failure of the M wave (muscle mass action potential evoked by single maximal shocks to the nerve) and a faster loss of force than occurred for the maximal voluntary contraction. A reduction in the stimulating frequency led to a restoration of both the M wave and the force output. As the motor neuron firing rate decrease during the maximal voluntary contraction correlated well with the rate of muscle contractile slowing, the authors suggested that this reduction in firing rate with fatigue served to optimise force output by allowing a relatively constant degree of tetanic fusion without electrical propagation failure.

The decrease in motor unit firing rate during fatiguing MVCs could therefore assist in maintaining optimal force output by avoiding peripheral neuromuscular transmission failure. However, a uniform decline in firing rates across motoneurons would not optimise force production by all muscle fibres as the contractile properties of motor units change differently with fatigue depending on their type [157]. The contractile properties of muscle fibres innervated by the same motor neuron are similar, while the contractile properties of muscle fibres within different motor units can be much more varied [65]. Dubose et al [112], for example, studied contractile activity of the cat medial gastrocnemius and found that twitch contraction time decreased in slow motor units with repeated contractions, while it increased in fast motor units.

The decrease in motor unit firing rates during sustained contractions could result from a combination of mechanisms. The central drive to the motor units could be altered, and excitatory and inhibitory systems linked to the central nervous system, the peripheral nervous system and the muscle could affect this drive. Gandevia [157] suggested that there is probably a reduction in spinal reflex facilitation and an increase in inhibition of motoneurons during isometric MVCs, making it more difficult to maximally drive the motoneurons by self-motivated volition. It has been suggested that the decline in motor neuron firing rate with fatigue is possibly caused by a reflex inhibition of motor neurons by afferents (Group III and IV) from the fatigued muscle [163,456], however a consensus has not yet been reached in this respect. Muscle spindle activity may also affect motor unit firing rates. Muscle spindles are recruited during voluntary isometric contractions and it has been suggested that they facilitate motor neuron discharge with fatigue [157,280]. Macefield et al [280] found that firing rate declined over time in most of the muscle spindle afferents tested during a fatiguing submaximal voluntary isometric contraction of the dorsiflexors. The authors hypothesised that the result of the decrease in spindle discharge would be a progressive disfacilitation of alpha-motoneurones, which could contribute to the decrease in motor unit firing rates evident during a fatiguing contraction.

4.2.2.9 Fatigue-stimulated muscle afferents

In order to respond to fatigue the nervous system needs to receive sensory information about the state of the muscle. While there are many plausible theories, the specific messengers that the motor control system responds to with different patterns of motor unit recruitment and firing frequency are not yet fully established [323]. Muscle spindles act by sensing the amount and rapidity of stretch in the muscle and sending afferent signals to the central nervous system [65]. Group III and IV muscle afferents respond to the local mechanical, thermal and biochemical conditions in the muscle [65,157]. They are activated with muscle contraction [65] and their discharge increases with fatigue [157]. This increase in activity with fatigue can result from many different factors, such as changes in lactic acid or potassium ion concentration, which will in turn depend on the length and level of contraction as well as muscle perfusion [99,378]. The group III and IV afferents probably exert their effects on the neuromuscular system in multiple ways, including presynaptic, spinal and supraspinal actions [157].

4.2.2.10 Supraspinal fatigue

Supraspinal fatigue is the "fatigue produced by failure to generate output from the motor cortex" [157]. Evidence for this supraspinal component to central fatigue is that force during a fatiguing trial can be increased from that generated voluntarily by the addition of transcranial stimulation of the motor cortex. Gandevia et al [158] stimulated the motor cortex of subjects performing a sustained isometric MVC to investigate motor output during fatigue. They found that the increase in force (from the voluntary force output level) produced by the magnetic cortical stimulation was small, but evident initially during the contraction, and increased with time during the sustained MVC. They concluded that during sustained MVCs, voluntary activation becomes sub-optimal such that stimulation of the motor cortex causes an increase in force output, and suggested that inadequate neural drive 'upstream' of the motor cortex is one of the sites involved in central fatigue.

Neuromuscular aspects of fatigue therefore involve both peripheral and central components, and the use of EMG and muscle stimulation can be used to clarify the contributions of these factors in the fatigue process during endurance activity. Many types of endurance activity, including distance running, involve elastic components within the neuromuscular system. This will now be discussed in relation to stretch-shortening cycle activity.

4.2.3 Stretch-shortening cycle muscle function

Human locomotion is not comprised of isolated isometric, concentric or eccentric contractions. Instead, normal human locomotion and most forms of exercise combine concentric and eccentric contractions of muscle groups in a temporal pattern during movement, known as the stretch-shortening cycle [39,239,250]. The stretch-shortening cycle (SSC) is a naturally-occurring pattern of movement that is evident in many types of locomotion and physical activity [39,250]. It involves the lengthening or stretching of a muscle as part of an eccentric contraction, followed by the shortening of that muscle during a concentric contraction [39]. For example, in running, the quadriceps muscles contract eccentrically during the initial loading and early midstance phase of the stride and then contract concentrically during the push-off phase. During a SSC movement, elastic energy appears to be stored in the activated muscle during the stretching phase, and used in the subsequent shortening phase, resulting in a potentiation of performance [178].

Optimising the force augmentation of the SSC would be desirable in sports that make common use of the SSC [39]. During running, the locomotory muscles repeatedly perform the SSC action during alternating gait cycles [208]. The storage and recovery of elastic energy in the muscle-tendon complex is therefore pertinent to running performance [131]. The muscles, tendons and ligaments in the leg have been described to act as a single linear spring during running [132]. Noakes [338] suggested that the more a muscle acts like a spring, the less energy it consumes and the more efficient it is. Efficient muscle will enhance performance by slowing the rate of accumulation of metabolites as well as the rate of rise of body temperature. The use of elastic energy in locomotion will therefore reduce the metabolic demands of the activity [9] and delay the progression of fatigue.

4.2.3.1 The functioning of the stretch-shortening cycle

The SSC is thought to work both through the use of elastic energy stored in the elastic components of the muscle during the stretch phase, as well as through reflex potentiation [49]. Reflex potentiation involves a stretch-induced increase in muscle spindle activity, which causes an increase in reflex input to the motor neurons and a subsequent increase in muscle activation. Bosco et al [49] found that the myoelectrical activity of the calf muscles was potentiated during the concentric phase of a counter-movement jump (includes a stretch phase) compared to a jump without a counter-movement (no stretch phase), and attributed the increase in performance to a combination of the utilisation of elastic energy and myoelectrical potentiation of muscle activation. Svantesson et al [424] also found that torque production during a concentric contraction was greater when preceded by an eccentric preload in the plantar flexors, and Helgeson and Gajdosik [187] reported a similar finding for the quadriceps muscles. Svantesson et al [424] reported that a concentric action preceded by an eccentric action generated a torque value on average about 100% larger than a concentric action alone. Unlike Bosco et al [49], however, they found that the muscles' EMG activity was lower or unchanged, suggesting elastic potentiation and not reflex potentiation was responsible for the increase in torque output. It is possible that these different findings are the result of the different testing protocols used. Bosco et al [49] used jump exercises performed on a force plate apparatus to investigate SSC performance, while Svantesson et al [424] used plantar flexion exercises on a dynamometer.

4.2.3.2 Factors enhancing stretch-shortening cycle function

There are a number of factors that contribute to the storage and utilisation of elastic energy. The degree of stretch during the eccentric contraction has been shown to be associated with SSC performance, such that prestretch load correlates positively with SSC efficiency [9,48,239,253]. Aura and Komi [9] suggest that with increasing prestretch, more activation takes place during the eccentric phase of a SSC activity and therefore more attached cross bridges will be stretched, so that the possibilities to use the muscle's elastic capacity will increase. They also contend, however, that the benefits to SSC performance gained by increasing prestretch are only valid up to a point, after which further increases in prestretch intensity no longer increase the mechanical efficiency of a SSC action. Kyröläinen and Komi [253] found that this effect of prestretch load on SSC activity was evident in both power and endurance trained athletes. In addition to the degree of prestretch affecting SSC performance, the velocity of prestretch has also been suggested to be of importance [48]. A high stretching velocity during the eccentric phase of a SSC movement facilitates a rapid increase in tendomuscular stiffness, which increases the force output during the concentric action [250].

The stretch-shortening cycle benefits derived from an eccentric contraction preceding a concentric contraction decay with the duration of the pause [452]. The transition time between the stretching and shortening phases of the movement is therefore of importance, with a short transition time between the eccentric and concentric actions associated with better storage and utilisation of elastic energy [48]. Aside from the timing and intensity of the eccentric and concentric movements, the composition of the musculotendinous tissue involved plays a role in SSC activity. The stiffness of tendon structures affects stretch-shortening cycle performance, possibly by affecting the storage and recoil of elastic energy [249]. Kubo et al [248] found that long distance runners performed worse in both squat and counter-movement jumps, and had a smaller difference in height between the two jump types than untrained individuals. They concluded that this difference in jumping ability could be related to the compliance of the muscle-tendon complex of the vastus lateralis and its potential for energy storage, both of which were lower in the distance runners than the untrained individuals. Wilson et al [453] studied male weight lifters performing a purely concentric bench press and a rebound bench press manoeuvre. They found that maximal series elastic component stiffness correlated significantly with the augmentation to the concentric movement that occurred when the rebound manoeuvre (involving prior stretch) was performed. They also found that the subjects performed the SSC portion

of the rebound bench press movement to coincide with the natural frequency of oscillation of their series elastic components.

The SSC action is apparent in natural movements such as running and jumping [194]. During these types of activity, the leg extensor muscles are pre-activated before contact with the ground. Kyröläinen et al [250] found that the gastrocnemius and the vastus lateralis muscles were "strongly preactivated" during the braking phase of take-off during a long jump, presumably to prepare the muscles to receive high impact forces. The authors suggested that this high level of pre-activation, together with a high level of muscle recruitment during the eccentric action and a high stretching velocity allow for a rapid increase in tendomuscular stiffness, which augments SSC performance and therefore jump performance. In this same study, the authors found high Achilles tendon forces in the push-off phase of running, despite low EMG activity in this phase. Their conclusion was that, in both long jump and running performances, muscle activity in the precontact and braking phase prepares the muscles for high impact loads and effective performance in the push-off phase.

4.2.3.3 Stretch-shortening cycle testing

SSC activity is commonly tested via a series of maximal, voluntary jumps during which EMG and force production are measured. Three jumps are generally performed: the squat jump (SJ) from a semi-squatting position with no countermovement; a countermovement jump (CJ) from a standing position with a preliminary countermovement, and a drop jump (DJ) from an elevated position onto the force plate with a subsequent jump off it. These three types of jump use different amounts of elastic energy and therefore allow investigation of the functioning of the SSC in subjects. Jump height or concentric force output during a CJ is generally higher than for a SJ [40,48,153,154,178,240]. Häkkinen et al [178] examined SSC jumps in elite weight lifters and found that the utilisation of stored elastic energy was observable during countermovement jumps performed at various loads. Subjects jumped higher during CJ's than during SJ's with no difference in the average quadriceps EMG activity between the concentric phases of the jumps. Bosco et al [48] found that the potentiation effect of the prestretch during the CJ was associated with a higher force at the end of prestretch as well as a high prestretch speed and a short coupling (transition) time.

Bobbert et al [40] also found that subjects could consistently jump higher when performing a CJ than a SJ. They suggested that, rather than this being the result of

increased storage and re-utilisation of elastic energy, that it resulted from the countermovement allowing the muscles to achieve a higher level of attached cross-bridges and force before the start of muscle shortening, which would enable the muscles to produce more work over the first part of the push-off. Fukashiro and Komi [153] also suggested that utilisation of elastic energy is not the only reason for the potentiation of performance of the CJ relative to the SJ. They found that, while the mechanical work of the knee extensors and ankle plantar flexors was similar for a SJ and a CJ, the work by the hip extensors was greater in the CJ, and therefore concluded that the performance difference between these jumps might result from the difference in work by the hip extensors.

As both the prestretch load and prestretch speed are likely to be greater in a DJ than a SJ (and indeed a CJ), it could be expected that SSC performance would be greater in a DJ than a SJ. Komi and Bosco [240] and Viitasalo and Bosco [440] found that subjects jumped significantly higher during a DJ than a SJ. Viitasalo and Bosco [440] divided subjects into two groups based on the muscle fibre distribution in their vastus lateralis and found that the relative height of rise of the centre of gravity for the DJ compared to the SJ was greater in the slow twitch muscle fibre group than the fast twitch fibre group. They suggested that it was therefore possible that the utilisation of elastic energy during jumping was better in subjects with a high percentage of slow twitch muscle fibres in their vastus lateralis muscles, although this finding should be interpreted with caution based on the small sample size (six subjects) of the study. Häkkinen et al [178] examined SSC activity in elite weight lifters, and in contradiction to the two previously mentioned studies, found no difference in jump height between DJ and SJ. The authors suggested that the reason their finding was in disagreement with other studies was possibly a result of the different training background of their athletes compared to the subjects in the other studies.

4.2.3.4 Effects of fatigue on the stretch-shortening cycle

Reduced storage of elastic energy during the stretch-shortening cycle activity during running has been implicated as one of the reasons for the decrease in performance with fatigue [349]. Stretch-shortening cycle performance has indeed been shown to be affected by fatigue. Horita et al [194] found that DJ performance decreased significantly immediately after, two hours after and two days after fatiguing SSC exercise. Gollhofer et al [172] found that when one hundred SSC movements were performed to induce fatigue, there was an increase in the duration of both the eccentric and the concentric phases of the SSC, and the transfer of energy between these two phases

was drastically reduced. Avela and Komi [10] found a decrease in maximal SSC performance after long-term SSC exercise and suggested that the reduction in performance was partly due to an impairment of the ability to utilise stiffness-related elastic energy, and not merely a direct effect of central or peripheral fatigue, although SSC-related fatigue processes could be considered to be a component of peripheral fatigue.

4.2.3.5 Stretch-shortening cycle and ethnicity

The effects of ethnicity on stretch-shortening cycle performance has not been well investigated. Black American athletes were found to have greater muscle viscosity and elasticity than white American athletes, but tendon elasticity was similar [152]. The black athletes therefore had greater muscle stiffness than the white athletes, which the authors suggested may be a result of a higher rate of cross-bridge cycling. Differences in muscle stiffness can affect stretch-shortening cycle performance, with greater muscle stiffness contributing to shorter ground contact time and faster push-off times during running and jumping, which generally results in better performances [152]. Variations in stretch-shortening cycle performance in African populations has not, however, been established.

One of the endurance running factors that reflects both muscle power and muscle elasticity is running stride. The role of stride parameters in endurance activity and fatigue will therefore now be discussed.

4.2.4 Stride parameters

The time a runner takes to cover a certain distance is determined by their stride length (SL) and stride frequency (SF). During running, part of the work results from the action of muscle-tendon 'springs' without metabolic cost and so work rate does not necessarily parallel metabolic rate with changes in speed or size [244]. Kram and Taylor [244] found that, at equivalent speeds, size-related differences in cost were proportional to stride frequency, suggesting that the time available for developing force was important in determining metabolic cost. They suggested that the primary determinants of the cost of running are therefore the cost of supporting the animal or person's weight and the time course of generating this force. Leifeldt et al [261] measured physiological parameters during treadmill running and found that, while oxygen consumption and blood lactate concentrations were lower during a downhill compared to a horizontal maximal treadmill run, maximal stride frequency was

similar. A higher stride length was reached on the downhill run and the subjects achieved a higher peak treadmill velocity. The authors proposed that, as similar maximal stride frequencies were reached during both types of run, factors involved in the rate of lower limb stride recovery may be limiting maximal running speed during these tests, rather than oxygen delivery. Deuel and Park [105] examined stride parameters of horses and found that superior performance in horseracing was associated with longer stride length. Stride frequency was most advantageous within a specific range, but optimal stride length had no upper limit. As stride kinematic patterns were associated with successful performance in horseracing, it is possible that this is also the case in humans.

4.2.4.1 Effects of stride parameters on running economy

Runners can adjust their SF and/or their SL to alter their running speed. Cavanagh and Kram [75] found that as speed increased from 3.15 $m.s^{-1}$ to 4.12 $m.s^{-1}$, SF remained nearly constant (4% increase) while SL increased by 28%. This data suggests therefore, that runners are more inclined to alter their running speed by changing their SL. Stride parameters can affect the economy of distance running [76]. Cavanagh and Williams [76] found that distance runners chose to run at a stride length that minimised their oxygen uptake, suggesting that the stride parameters adopted by a runner may be those that incur the lowest metabolic cost. Farley et al [131] found that when subjects hopped at their preferred frequency or higher, their bodies behaved like a simple spring-mass system. At frequencies below the preferred frequency, however, their bodies did not behave in this spring-like manner, which would presumably result in a reduction in the storage and recovery of elastic energy and therefore be less economical.

While it could be assumed that the most economical stride parameters would be dependent on an individual's size and shape, Cavanagh and Kram [75] found that anthropometric variables (such as stature and leg length) did not correlate significantly with preferred SL in distance runners. They suggested that factors other than anthropometrical variables are the primary determinants of preferred SF and SL. During running, leg stiffness is adjusted to accommodate changes in SF, such that muscle stiffness increases with greater stride frequencies [132,133]. During a study on hopping, Farley and Morgenroth [133] found that the primary mechanism for adjustment of leg stiffness was the adjustment of ankle stiffness, rather than knee stiffness.

4.2.4.2 Effects of fatigue on stride parameters

Stride frequency appears to be a repeatable day-to-day measure in well-trained runners [55]. However, it can be affected by fatigue. Gollhofer et al [172] found that when intense SSC activity was performed to induce fatigue, times for both the eccentric and concentric phases of the SSC increased. An increase in ground contact time during running would in turn affect the SF. Sharwood [401] compared stride parameters during a 20 m sprint before and after a fatiguing five km time trial. It was found that, with fatigue, SF and SL were reduced and the ground contact time for each stride was increased, which resulted in a slower sprint performance.

4.2.5 Summary

Performance in endurance activities may be limited by factors related to the force and velocity characteristics of the neuromuscular system, including neural control of muscle force production and the capability to store and utilise elastic energy. Muscle force production is affected by neural, mechanical and muscle factors, and the level of force output can be altered by changing the number of active motor units, the type of muscle fibres recruited and the motor unit firing rate. Neural activation and the resulting muscular action are different between different types of contraction, and neuromuscular fatigue profiles therefore also vary for isometric, concentric and eccentric muscle activity. The mechanisms that result in fatigue involve all components of the motor system, from a failure of or reduction in the production of the descending drive to a decrease in contractile protein activity. Fatigue is therefore often broken down into central fatigue, defined as "a progressive reduction in voluntary activation of muscle during exercise" and peripheral fatigue, namely "fatigue produced by changes at or distal to the neuromuscular junction" [157]. The neural recruitment of muscle is one of the potential sources of racial differences in athletic performance.

Measurement of skeletal muscle motor unit recruitment, and therefore measurement of the electrical manifestations of fatigue phenomena in the neuromuscular system, can be performed using electromyography. Analysis of the EMG signal to gain insight into muscle activity often involves the calculation of the amplitude and the frequency content of the power spectrum of the electromyogram. EMG amplitude and frequency are dependent on the number and type of motor units active as well as their firing rates. These variables changes with fatigue differently depending on the type of contraction being performed and the intensity of the contraction. Peripheral and

central fatigue during or after a fatiguing contraction is also examined by observing the effects of electrical muscle stimulation on force output. In order to respond to fatigue the nervous system needs to receive sensory information about the state of the muscle. Many factors play a role in this, but the full host of messengers that the motor control system responds to is not yet fully established.

The stretch-shortening cycle is a naturally-occurring pattern of movement involving the lengthening or stretching of a muscle during an eccentric contraction, followed by the shortening of that muscle during a concentric contraction. Optimising the force augmentation of the SSC would reduce the metabolic demands of an activity and delay the progression of fatigue. The SSC is thought to work both through the use of elastic energy stored in the series elastic components of the muscle during the stretch phase, as well as through reflex potentiation. A number of factors contribute to the storage and utilisation of elastic energy, including the degree and velocity of prestretch, the transition time between the stretching and shortening phases, the composition of the musculotendinous tissue and the level of pre-activation. Reduced storage of elastic energy during SSC activity is probably one of the reasons for the decrease in performance with fatigue during activities such as endurance running. The time a runner takes to cover a certain distance is determined by their stride length and their stride frequency, which are affected by muscle elasticity and stiffness. These stride parameters can affect the economy of distance running, and can be affected by fatigue. The effects of ethnicity on SSC performance have not been well investigated.

4.3 INTRODUCTION

Endurance performance is affected by many components of the neuromuscular system, including both neural and muscular factors as well as the use of elastic energy [349]. The neuromuscular system therefore plays a central role in the resistance of fatigue, as is evident in the decreased physical endurance that accompanies many neuromuscular pathologies [143,259].

Neuromuscular causes of fatigue and neuromuscular factors associated with endurance performance have been fairly widely researched, as described in the literature review. In particular, neuromuscular studies are conducted to elucidate the relative roles of central and peripheral factors in fatigue [31,158,227,232,258,274,275,349,395,413]. The extent of the contributions of central and peripheral fatigue during different types of exercise, however, remains controversial [157]. By understanding the neuromuscular factors involved in fatigue, methods of delaying or minimising the adverse effects of fatigue can be formulated.

Neuromuscular characteristics could also be related to the difference in athletic performance evident between different ethnic populations [7,84,152]. Coetzer et al [84] found greater fatigue resistance in black compared to white South African runners during repetitive isometric knee extensions. However, they did not measure changes in neuromuscular variables during the fatiguing trial and therefore could not determine potential sites of fatigue. Our study will therefore include a similar fatigue trial, incorporating electromyographic measurements into the protocol.

This chapter will therefore examine muscle strength, muscle endurance and muscle elasticity, along with the neuromuscular recruitment patterns associated with these. The neuromuscular variables will be analysed for the complete group of subjects and related to running performance to identify neuromuscular factors that are associated with endurance ability. The results will also be compared between black and white South African runners in order to identify ethnic differences in these variables and relate them to running performance differences in the South African population.

Maximal muscle strength tests will be performed with the quadriceps muscle group, as this muscle group is one of the most important in running, and indeed many forms of exercise. A knee extension movement will be used, as this is a single joint movement and so is relatively simple for study. Isometric, concentric and eccentric

strength tests will be performed in order to determine which, if any, of these are related to endurance performance, and how they differ between ethnic populations.

Maximal and submaximal isometric knee extension fatigue tests will be performed. The neuromuscular fatigue responses to these two intensities of contraction are different [137] and therefore both are relevant for investigation. An isometric knee extension will be used, as exercise using only one muscle group simplifies the accurate measurement of motoneuronal drives with electrophysiological techniques, and the neural mechanisms involved can be more easily described for isometric than dynamic exercise [157]. Stretch-shortening cycle testing will be used to investigate the relationship between elastic energy utilisation and endurance performance, as well as to determine whether or not the stretch-shortening cycle is functioning differently in the different ethnic populations. Stride parameters will also be examined as this may affect running economy.

The neuromuscular patterns during the stretch-shortening cycle tests and the neuromuscular causes of fatigue during the fatigue tests will be discussed, along with the relative roles of central and peripheral fatigue.

4.4 METHODS

4.4.1 Subject characteristics

The subject characteristics were previously described in section 2.4.1 of Chapter 2. All 32 subjects were initially analysed together as a single group (Part A), and subsequently analysed as a group of 16 black runners and a group of 16 white controls (Part B). The reason for dividing the groups in this manner is described in the subject selection section of the thesis introduction (section 1.3).

4.4.2 Experimental design

The details of the experimental design were previously described in section 2.4.2 of Chapter 2. The design is also outlined in Figure 2.1 of Chapter 2. During the first visit subjects performed a vertical jump test (section 4.4.6). On the second visit subjects performed muscle strength tests and isometric fatigue tests on a dynamometer (section 4.4.4), followed by stretch-shortening cycle (SSC) jump tests on a forceplate apparatus (section 4.4.5). Surface electromyographic (EMG) measurements were taken during the dynamometer testing and the SSC jump testing (section 4.4.3). On the third visit subjects performed an interval run (as described in Chapter 2, section 2.4.4.4), during which stride frequency measurements were taken (section 4.4.7).

4.4.3 Electromyographic (EMG) testing and muscle stimulation

4.4.3.1 Electrode placement:

Electromyographic readings were taken from the rectus femoris muscle of the right thigh. EMG measurements from this muscle have previously been shown to be reliable [438]. The subjects' skin was prepared by shaving off the hair, removing the outer layer of epidermal cells with sand paper and cleaning the skin with alcohol swabs. This facilitated electrode adherence and conduction of the EMG signal. After preparation of the skin, a surface Triode EMG electrode (Thought Technology Triode™ MIEP01-00, Montreal, Canada) was secured on the skin over the belly of the rectus femoris muscle, aligned parallel to the underlying muscle fibres [228]. The electrode was not moved throughout testing. The electrode was linked to an amplifier box, which was in turn linked via fibre optic cable to a computer with Flexcomp/DSP software (Thought Technology, Montreal, Canada). Two electrodes for electronic percutaneous muscle stimulation (Fuji System) were secured on either side of the recording electrode, above and below the belly of the muscle. These were linked via wire cable to an output jack on the stimulator box.

4.4.3.2 Signal processing:

The raw EMG signal was amplified and low pass filtered at a frequency of 500 Hz. The EMG was sampled at 1984 Hz, a frequency high enough for reliable data collection and quantitative data analyses [197]. The sampled EMG was passed through a 50 Hz line filter to remove interference from electrical sources to yield raw data. Movement artifact was removed from the raw signals with a high-pass second order Butterworth filter with a cut off frequency of 15 Hz (for the stretch-shortening cycle jump tests) or 5 Hz (for the isometric fatigue tests). The means of the EMG signals were then removed and the signals were full wave rectified. The signals were smoothed with a linear envelope using a low-pass second order Butterworth filter with a cut off frequency of 5 Hz. Filtering procedures were performed using MATLAB™ software (The MathWorks Inc., Natick, MA).

EMG recordings were taken during all of the muscle strength tests and fatigue tests performed on the dynamometer, and during all of the stretch-shortening cycle jump tests. Surface EMG has been shown to be a reliable method for studies of the neuromuscular system, in particular during stretch-shortening cycle activity [171]. Out of the four 5 s isometric maximal voluntary contractions, EMG was only analysed for the one that yielded the greatest force output. Half a second of EMG data was sampled from the centre period (from 2.25 s to 2.75 s) of the EMG collected during this contraction. In a similar manner, 0.25 s of EMG was sampled from the centre period of both the eccentric and the concentric contraction that yielded the greatest torque. A total of 0.5 s of EMG was sampled from the EMG recorded during each of the SSC tests. This was comprised of two 0.25 s samples, one taken from the eccentric phase of the contraction and one from the concentric phase. During the squat jump only 0.25 s was sampled due to the contraction only having an eccentric phase. The EMG from the isometric fatigue tests was broken down into 0.5 s epochs for analysis as will be detailed below with the description of time normalisation. For all tests the filtered EMG data was processed to yield amplitude and frequency compression data using specifically developed software [325,326]. EMG amplitude was calculated using the root mean squared (RMS) method, while frequency compression was calculated as mean percentile frequency shift according to the method of Lowery et al [277] as a modification of the work of Lo Conte and Merletti [267] and Merletti and Lo Conte [308].

4.4.3.3 EMG normalisation:

Normalisation of data was performed by expressing the isometric EMG data (maximal fatigue test and submaximal fatigue test) from each subject relative to the EMG from the 5 s isometric maximal voluntary contraction (MVC) for that same subject. The stretch-shortening cycle data was normalised by expressing the EMG from the eccentric part of the jump relative to the EMG from the maximal eccentric contraction and expressing the EMG from the concentric part of the jump relative to the EMG from the maximal concentric contraction. As the squat jump only had a concentric component it was only expressed relative to the maximal concentric contraction. These data were described as normalised EMG. By normalising the subjects' EMG values relative to their own maximal value (and not merely working with raw EMG data), potential effects of differences in lean muscle mass and body fat in the subjects were negated. Normalised muscle recruitment data is expressed as a percentage of maximum.

The maximal and submaximal isometric fatigue tests were of different lengths for all the subjects as a result of them maintaining the contraction for different lengths of time. The data was therefore analysed by breaking the entire EMG recordings into 0.5 s epochs, extracting the amplitude and frequency information, normalising this to the isometric MVC, and then time normalising the resulting data points by simple averaging [326]. The time normalisation procedure resulted in 10 separate periodogram estimates for 10 distinct periods of equal length between the start and end times of the test. This method of data processing and time-normalisation is advantageous in that the analysed epoch size remains constant at one half-second, maintaining the assumptions of EMG stationarity [36] as well as the fact that all the data is included in the analysis (as opposed to selecting, for example, 10 spaced half-second epochs). Most importantly, expressing the fatigue test EMG results over ten datapoints in this manner allowed statistical comparison of the data between individuals and groups.

4.4.3.4 Muscle stimulation:

Using the electronic muscle stimulator (EMS/400^{TM}, Fuji System), the subjects' rectus femoris muscle was externally stimulated to contract for one second, using a stimulation pulse rate of 64 Hz was and a current of 50 mAmp. This stimulation was performed both 15 seconds into and immediately before the end of the submaximal isometric muscle fatigue test. The second stimulation was administered when the subject indicated that they could contract no longer and were about the stop the

effort, but before the subject relaxed the contraction (this protocol was clearly explained to the subjects before they began the test).

4.4.4 Muscle strength

Isokinetic and isometric knee extensor muscle strength tests were performed using a Kin-Com Dynamometer (Chattanooga Group, Inc., Chattanooga, USA). The subjects were seated with their hips in 90 degrees flexion and were stabilised in the chair with a strap across the chest and waist. They were asked to keep their arms folded across their chest to prevent use of their upper body during leg extension, as hand-grip stabilisation can increase torque output of the knee extensors in men [421]. Isometric tests were performed with the knee in 60 degrees flexion (0 degrees being the limb in full extension) and isokinetic tests were performed with the knee flexing between 6 and 84 degrees at a speed of 60 degrees per second. The subjects warmed-up prior to isokinetic testing with four concentric and four eccentric contractions at 50% of their subjective maximum, two at 70% of maximum and one at 90% of maximum capacity. The warm-up for the isometric tests followed the same pattern, with five-second isometric contractions at 50%, 70% and 90% of subjective maximum.

For the isokinetic protocol, the subjects completed three concentric and three eccentric maximal contractions. From these, the concentric and the eccentric contraction with the greatest torque were selected for analysis. This allowed for determination of the subjects' maximal isokinetic torque output and normalisation of their stretch-shortening cycle EMG data. The isometric testing protocol consisted of three different tests. The first required subjects to perform four 5 s isometric maximal voluntary contractions (MVC's), from which the one with the greatest force output was selected for analysis. This allowed for both the determination of the subjects' maximal isometric force output and normalisation of their fatigue test EMG data. The second isometric test required subjects to complete a maximal voluntary isometric leg extension until task failure (until they relaxed the contraction). Subjects were instructed to push as hard as possible from the start of the test and to keep pushing as hard as they could for as long as they could. The third isometric test required subjects to perform a leg extension contraction at 20% of maximal force output (calculated from the peak force achieved during the 5 s maximal tests) until task failure (until they relaxed the contraction or let their force output fall below the 20% level three times). The subjects were verbally encouraged throughout the tests to exert their maximum effort. All subjects were encouraged with equal enthusiasm.

4.4.5 Stretch-shortening cycle (SSC) jumps

Stretch-shortening cycle jumps were performed in order to investigate force generation utilising elastic energy. The subjects performed a squat jump, counter-movement jump and drop jump on a forceplate (Advanced Mechanical Technology Inc., 6 channel forceplate, Massachusetts, USA) similarly to the method previously described by Komi and Bosco [240]. To perform the squat jump (SJ) and counter-movement jump (CJ) the subjects began by standing on the forceplate, then bent their knees to approximately 90 degrees and jumped off the forceplate in an upward and forward direction. When performing the CJ the subjects jumped as soon as their knees were bent to approximately 90 degrees, while in the SJ this squatting position was held for two seconds before jumping. The stretch-shortening cycle was therefore activated in the CJ, but not the SJ. To perform the drop jump (DJ) the subjects began by standing on a 49 cm high bench placed 30 cm away from the forceplate, then stepped off the bench to land with both feet on the forceplate, immediately bent their knees to approximately 90 degrees and jumped off the forceplate as in the CJ. The subjects practised each jump before the test to familiarise themselves with the technique and to allow the investigators to correct them if necessary. Each type of jump was performed three times with the subjects' hands clasped behind their backs. The subjects were instructed to jump from the forceplate with maximal effort.

The ground reaction forces generated on the forceplate were recorded for all SSC jumps. The mean and peak vertical force generated during the push-off (concentric) phase of each of the jumps was calculated. The push-off phase of the ground reaction forces was determined by the orientation of the horizontal force [250]. As each subject performed three SJ's, CJ's and DJ's, the force values obtained from each of the these was averaged for each jump type to yield a mean and a peak vertical force value for each subject. The difference between the force values from the concentric phase of the CJ and the SJ, the DJ and the SJ as well as the DJ and the CJ were calculated to determine the amount of additional force generated during push-off through stretch-shortening cycle energy. In addition, for each subject, the mean force generated during the concentric phase of each jump type was divided by the normalised EMG value obtained for the concentric phase of the same jump type to yield a force/EMG value. This ratio is described as 'neuromuscular efficiency' and indicates the amount of force generated 'per unit EMG' or 'per unit muscle activity' during the push-off phase of the jump.

4.4.6 Vertical jump

For the purpose of marking jump height, the subjects covered their fingers in chalk powder. The subjects then stood next to a wall-mounted chalkboard, stretched straight upwards and made a mark with their hand on the chalkboard. They then jumped vertically upwards from a standing position, making another mark on the chalkboard at the peak height of their jump. The vertical distance between their standing mark and their jump mark represented their vertical jump height. They performed the jump three times and their highest jump was used for subsequent analysis.

4.4.7 Stride parameters

Stride frequency (SF) was measured while the subjects were running at 12 km/hr on a treadmill during an interval run (in which subjects ran at 10, 12, 14 and 16 km/hr, in order of increasing speed; refer to Chapter 2, section 2.4.4.4). SF was measured by counting the number of steps taken with the subject's right leg in one minute. This was used to calculate stride length (SL) in metres at 12 km/hr with the equation: SL = distance in metres covered in 1 minute/SF = (12000/60)/SF = 200/SF.

4.4.8 Statistical analysis

Statistical analyses were performed using the Statistica software package (Version 6, Statsoft, Tulsa, OK, USA). Correlations between physiological variables and performance variables in part A were performed with the Pearson Product-Moment Correlation. Comparisons of SSC jump variables in part A were performed using the paired Students' t-test. The change in EMG variables with time in part A (time effect), and the difference in the change in EMG variables over time between the two groups in part B (interaction effect), were performed using a repeated measures ANOVA. Where significance was identified using the repeated measures ANOVA, the Tukey HSD Post-hoc test was used to identify differences between individual time points (part A) or differences between group means at the individual time points (part B). When the data was divided into two groups based on ethnic origin (part B), comparisons of variables between the two groups were performed using the unpaired Students' t-test. Statistical significance was accepted when $p<0.05$. Complete sets of data were not always collected for all subjects during the strength tests, the fatigue tests and the jump tests as a result of equipment malfunction (this included dynamometer load cell malfunction, EMG signal interference, forceplate electrical error and computer system hard drive error). This missing data was therefore

excluded from statistical analysis, as reflected in the subject number (n) values in the results section of this chapter.

4.5 RESULTS

4.5.1 Part A: Physiological variables and endurance performance

4.5.1.1 Muscle strength

The subjects' peak quadriceps isometric force output was 537 ± 166 N, while their peak concentric and eccentric torque output were 158 ± 44.6 Nm and 229 ± 65.0 Nm, respectively. Neither peak isometric force output nor peak concentric or eccentric torque output correlated significantly with 10 km personal best time (PB), whether the force variables were expressed as absolute values, per body mass or per lean thigh volume (LTV, Table 4.1).

Table 4.1: Correlation of the peak isometric force output and peak concentric or eccentric torque output with 10 km personal best time, with the force variables expressed as absolute values, per body mass or per lean thigh volume (LTV) (n=26).

	Isometric force		Concentric torque		Eccentric torque	
	r value	p value	r value	p value	r value	p value
Absolute value	0.0836	0.685	-0.0734	0.721	-0.1768	0.387
Per body mass	-0.0257	0.901	-0.2629	0.194	-0.3606	0.070
Per LTV	0.3539	0.076	0.2077	0.309	0.0429	0.835

4.5.1.2 Isometric fatigue

The runners' time to task failure (TTF), or 'fatigue', was 97.6 ± 78.2 s for the maximal isometric fatigue test and 233 ± 97.6 s for the submaximal isometric fatigue test. TTF correlated significantly with 10 km PB for the maximal fatigue test ($p<0.05$), but not for the submaximal test (Figure 4.1).

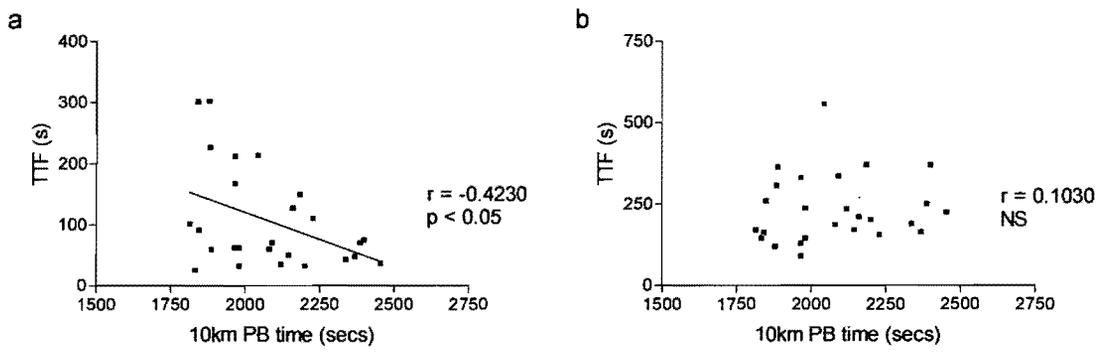


Figure 4.1: Correlation of 10 km personal best time (PB) with time to task failure (TTF) during the maximal (a) and submaximal (b) isometric fatigue tests (n=26).

The subjects' quadriceps EMG amplitude changed significantly with time during the maximal isometric fatigue test ($p<0.001$), decreasing by 24.6 ± 31.8 % from time period one to ten (Figure 4.2 a). This difference was significant from time period six. The subjects' EMG frequency also changed significantly with time during the maximal fatigue test ($p<0.001$), decreasing by 15.6 ± 11.1 % from time period one to ten (Figure 4.2 b), with the difference significant from time period two. In contrast to the maximal fatigue test, during the submaximal isometric fatigue test, the subjects' quadriceps EMG amplitude changed significantly with time ($p<0.001$), increasing by 14.3 ± 20.2 % from time period one to ten (Figure 4.2 c). This difference was significant from time period eight. The subjects' EMG frequency also changed significantly with time during the submaximal fatigue test ($p<0.001$), decreasing by 5.45 ± 8.21 % from time period one to ten (Figure 4.2 d), with the difference significant from time period seven.

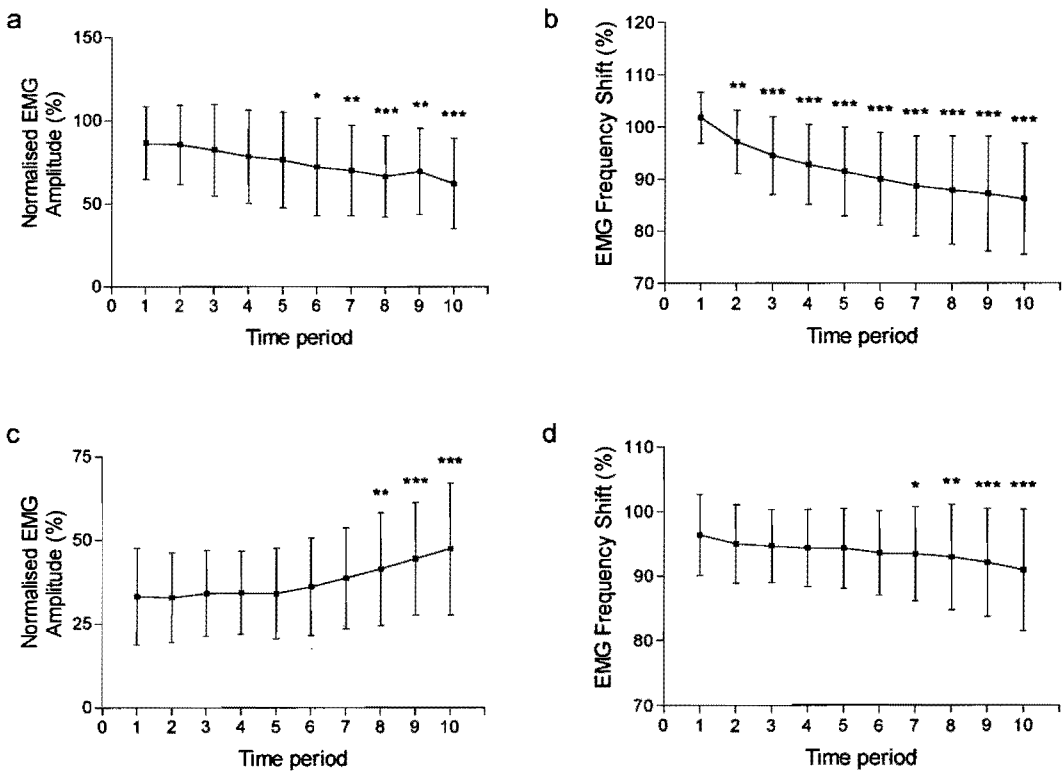


Figure 4.2: Electromyographic changes with time during quadriceps isometric fatigue tests, namely: EMG amplitude (a) and frequency shift (b) during the maximal fatigue test (n=30), and EMG amplitude (c) and frequency shift (d) during the submaximal fatigue test (n=31). *'s indicate a significant change from time period 1. *$p<0.05$; **$p<0.01$; ***$p<0.001$

An electrical muscle stimulation was applied to the subjects' quadriceps both near the beginning of the submaximal fatigue test and at the end. The first stimulation increased the subjects' force output by an additional 152 ± 79.4 % from the submaximal force output, while the second increased the force output by 82.3 ± 68.0 %. There is a significant difference of 69.5 % between these values ($p<0.001$). An example of the force output data during this test from one of the subjects is shown in Figure 4.3. This decrease in stimulated force output between the first and second stimulation did not correlate significantly with TTF during the submaximal fatigue test ($r=0.0463$, $p=0.822$).

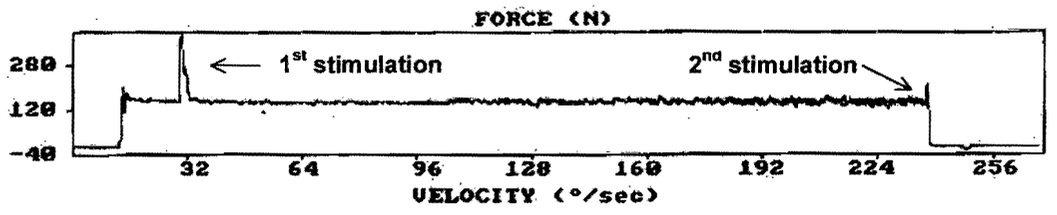


Figure 4.3: An example of the force output during the submaximal quadriceps isometric fatigue test from one of the subjects, showing the increase in force output with the muscle stimulations. There is an increase in force output for both the 1st and the 2nd stimulation, although the increase is less for the 2nd.

4.5.1.3 Stretch-shortening cycle muscle function

The mean vertical force output generated during the push-off (concentric) phase of the counter-movement jump (CJ) was significantly greater than that during the squat jump (SJ, $p<0.001$), and the mean force from the drop jump (DJ) was significantly greater than that from the SJ ($p<0.001$) and the CJ ($p<0.001$, Figure 4.4). The peak force output (N) during the push-off phase of the stretch-shortening cycle jumps showed a similar result to the mean force output, with significantly greater force generated during the DJ than both the SJ ($p<0.001$) and the CJ ($p<0.001$). However, there was no significant difference in peak force output between the SJ and the CJ.

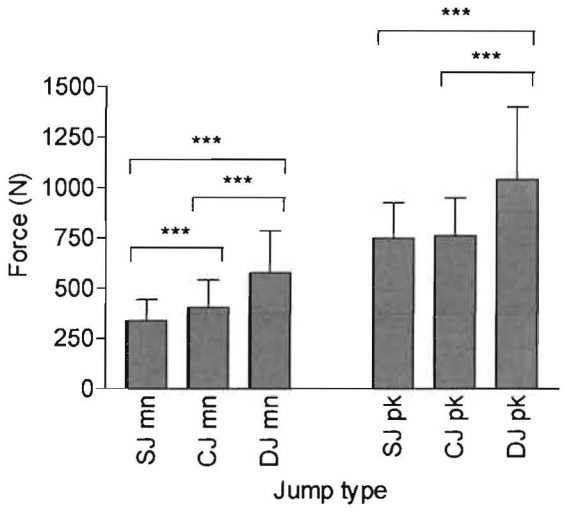


Figure 4.4: Mean (mn) and peak (pk) force output (N) during the push-off (concentric) phase of the squat jump (SJ, n=28), counter-movement jump (CJ, n=28) and drop jump (DJ, n=29). *'s indicate a significant difference between the force values for the jump types. ***p<0.001

The EMG amplitude from the concentric phase of the SJ was significantly greater than that of both the CJ ($p<0.05$) and the DJ ($p<0.05$, Figure 4.5). There was, however, no significant difference between the CJ and the DJ for the EMG amplitude from the concentric or eccentric phase.

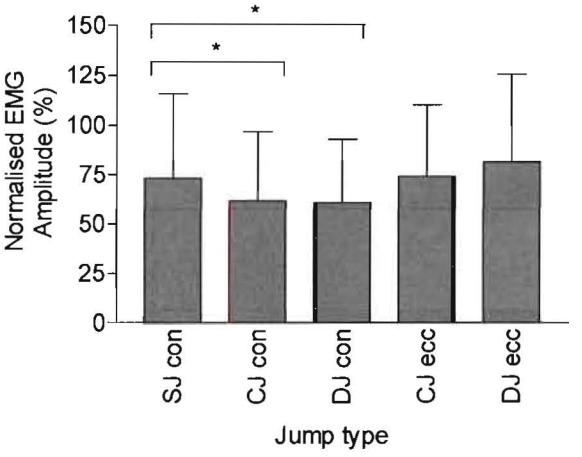


Figure 4.5: EMG amplitude during the concentric (con) and eccentric (ecc) phases of the squat jump (SJ), counter-movement jump (CJ) and drop jump (DJ) (n=30). *'s indicate a significant difference between the different types of jump. *p<0.05

The push-off, or concentric, phase of the CJ and the DJ were significantly more efficient (mean force output per normalised EMG amplitude) than that of the SJ ($p<0.01$ and $p<0.05$, respectively, Figure 4.6). There was no significant difference in neuromuscular efficiency between the CJ and the DJ, although the comparison neared statistical significance ($p=0.050$). The stretch-shortening cycle delta values for the difference in neuromuscular efficiency between the SJ and the CJ ($r=0.1493$ and $p=0.497$), the SJ and the DJ ($r=0.3069$ and $p=0.145$), and the CJ and the DJ ($r=0.3025$ and $p=0.151$) did not correlate significantly with 10 km PB.

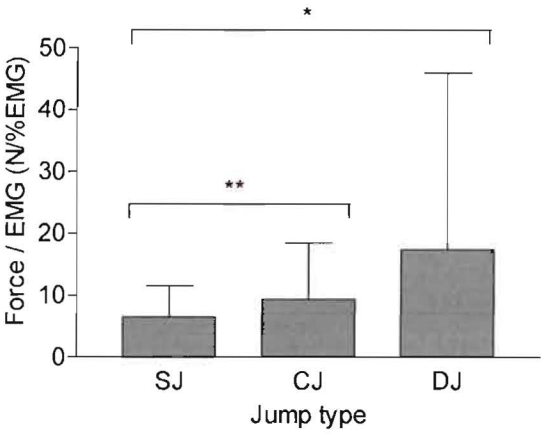


Figure 4.6: Neuromuscular efficiency (mean force output per normalised EMG amplitude (N/%EMG)) during the push-off (concentric) phase of the squat jump (SJ, n=27), counter-movement jump (CJ, n=27) and drop jump (DJ, n=28). *'s indicate a significant difference between the efficiency values for the jump types. *$p<0.05$; **$p<0.01$

The mean vertical jump height reached by the subjects was 42.2 ± 5.46 cm. While vertical jump height did not correlate significantly with the difference in neuromuscular efficiency between the SJ and the CJ ($r=0.1971$ and $p=0.356$) or the SJ and the DJ ($r=0.3901$ and $p=0.054$), it was positively correlated with the difference in neuromuscular efficiency between the CJ and the DJ ($r=0.4272$, $p<0.05$). The subjects' mean vertical jump height did not correlate significantly with their 10 km PB ($r=0.0268$ and $p=0.897$).

4.5.1.4 Stride parameters

The subjects' mean stride length when running at 12 km/hr was 2.40 ± 0.13 m. There was a significant negative correlation between stride length at 12 km/hr and PB (Figure 4.7 a, $p<0.05$), i.e. the longer the strides taken when running at a submaximal speed, the quicker a 10km race is run. The stride length data was then expressed relative to the subjects' heights and this ratio correlated with the performance

variables, to determine if the distance a runner covers with each stride relative to his height was associated with running performance. This correlation was also significant (Figure 4.7 b, $p<0.01$), indicating that the further a runner travels with each step (expressed relative to his height) when running at 12 km/hr, the faster he runs a 10 km race.

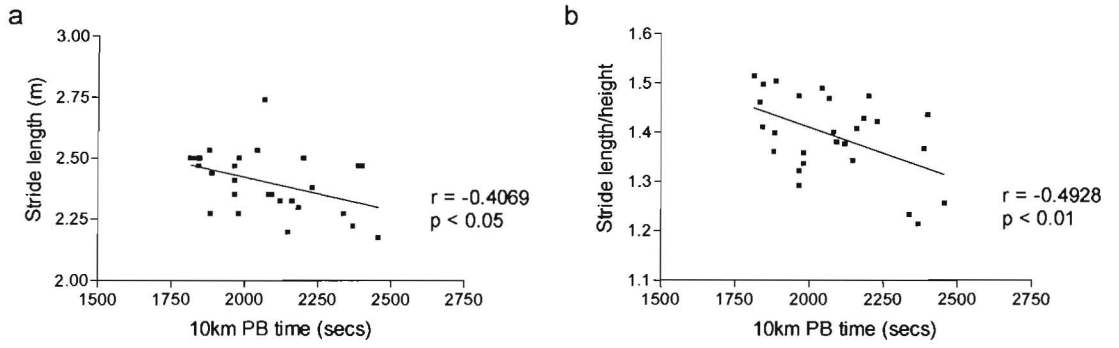


Figure 4.7: Correlation of stride length (at 12km/hr, a) and stride length/height (b) with 10km personal best time (PB, n=27).

4.5.2 Part B: Ethnic comparison

4.5.2.1 Muscle strength

The peak isometric quadriceps force output was greater in the white than the black runners ($p<0.001$), as was the peak concentric torque output ($p<0.01$, Figure 4.8 a and b). The peak eccentric torque output, however, was not significantly different between the groups. When the isometric force output and isokinetic torque output were expressed per body mass, however, there were no significant differences in the peak values between the groups (Figure 4.8 c and d). Expressing the quadriceps force and torque values per lean thigh volume (LTV), however, yielded similar results to the absolute values (Figure 4.8 e and f). The peak isometric force/LTV ($p<0.001$) and peak concentric torque/LTV ($p<0.01$) were greater in the white runners than the black, while there was no difference between the groups for peak eccentric torque output.

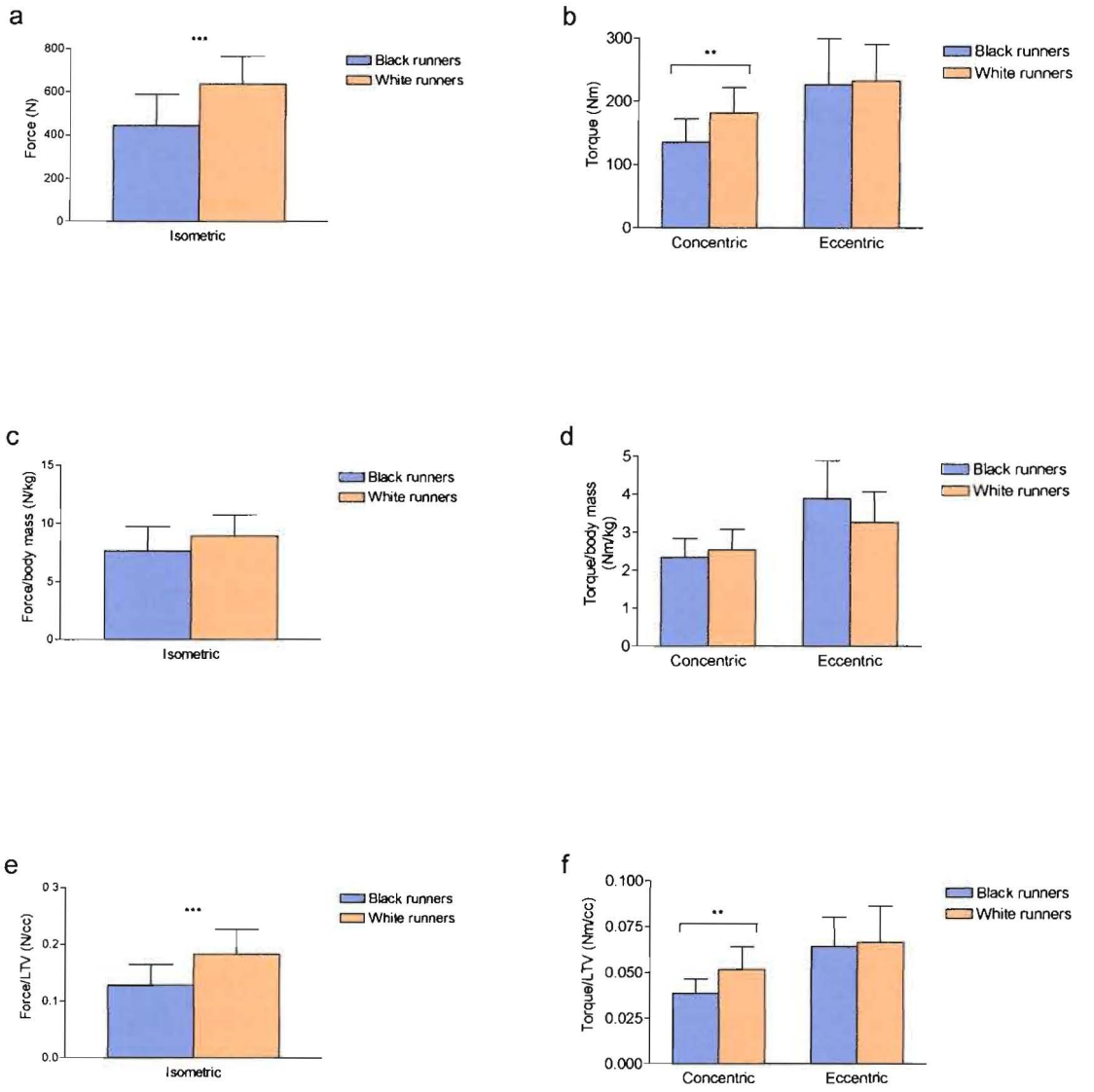


Figure 4.8: Peak isometric and isokinetic quadriceps force and torque output of black (n=16) and white (n=15) runners, expressed as absolute values (a, b), per body mass (c, d) and per lean thigh volume (LTV) (e, f). *'s indicate a significant difference between black and white groups. **p<0.01; ***p<0.001

4.5.2.2 Isometric fatigue

The TTF during the maximal isometric quadriceps fatigue test was not significantly different between the black and white runners (Figure 4.9). The TTF during the submaximal fatigue test was, however, significantly different between groups, with the black subjects lasting a mean of 76 s longer than the white ($p<0.05$, Figure 4.9).

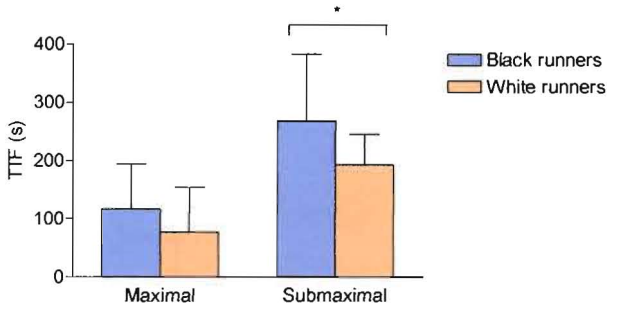


Figure 4.9: Time to task failure (TTF) of black and white runners during a maximal (black n=16; white n=15) and a submaximal (black n=16; white n=14) isometric quadriceps contraction. *'s indicate a significant difference between black and white groups. *p<0.05

There was no significant difference between the black and white runners for the change in quadriceps EMG amplitude with time during the maximal isometric fatigue test (Figure 4.10 a). There was also no significant difference between the groups for the change in EMG frequency with time during the maximal fatigue test (Figure 4.10 b). There were, however, electromyographic differences between the groups for the submaximal isometric quadriceps fatigue test. During this test, the EMG amplitude increased significantly more over time in the white subjects than it did in the black ($p<0.05$, Figure 4.10 c), while the EMG frequency decreased more over time in the white subjects than it did in the black ($p<0.001$, Figure 4.10 d).

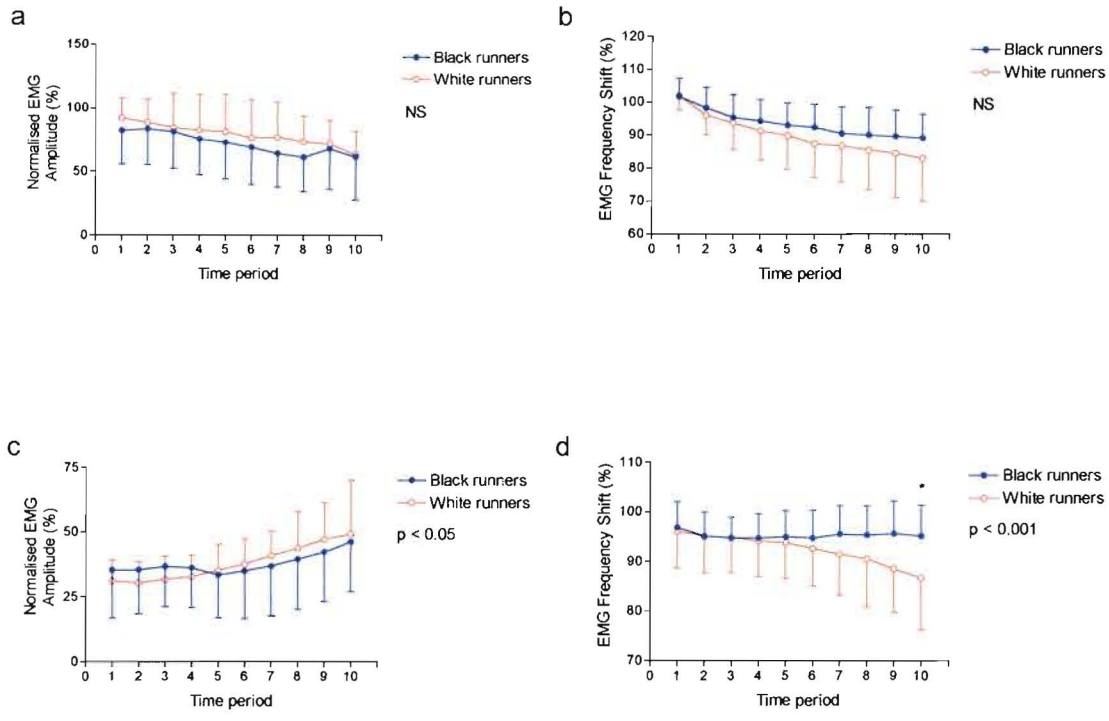


Figure 4.10: Electromyographic changes with time (expressed as ten normalised time periods) during quadriceps isometric fatigue tests in the black and white runners, namely: EMG amplitude (a) and frequency shift (b) during the maximal fatigue test (black n=16; white n=14), and EMG amplitude (c) and frequency shift (d) during the submaximal fatigue test (black n=16; white n=15). The given p value indicates a significantly different interaction effect (EMG change over time) between black and white groups. *'s indicate a significant difference at a particular time point between black and white groups. *p<0.05

The EMG data for the maximal and submaximal isometric fatigue tests is shown again in Figure 4.11, but with the time values on the x axis not normalised, to demonstrate the difference in EMG between the groups in 'real time' over the course of the fatigue trials. No statistical comparisons were performed on this data, as the values for the two groups could not be compared in the non-normalised state.

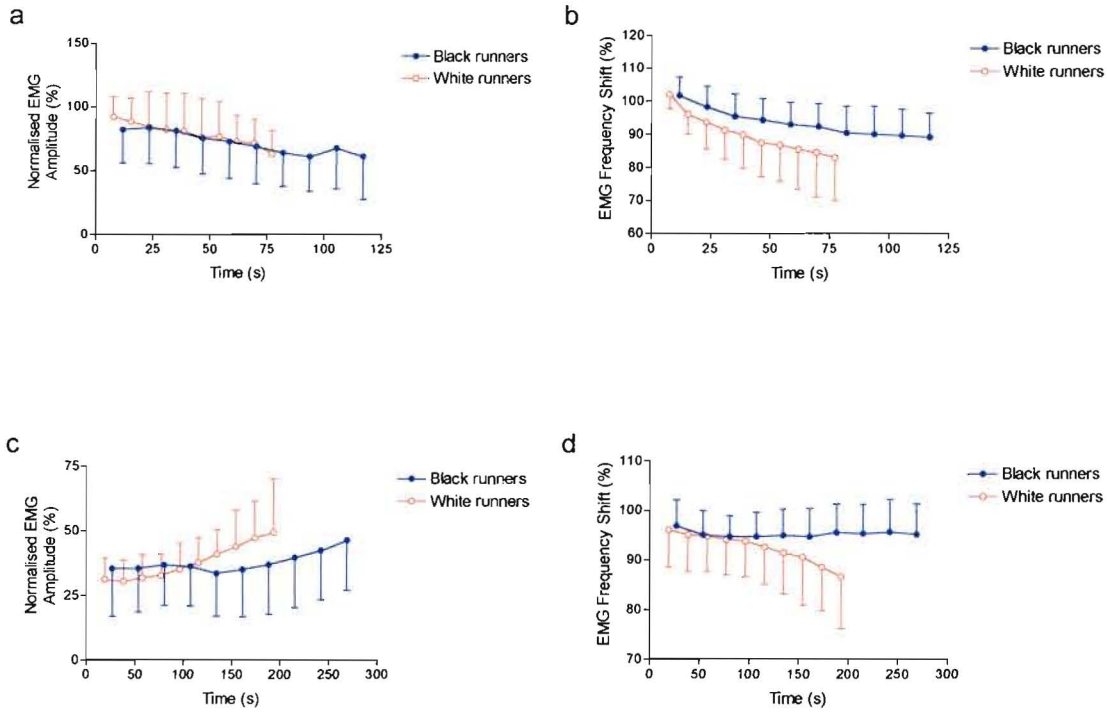


Figure 4.11: Electromyographic changes with time (in seconds) during quadriceps isometric fatigue tests in the black and white runners, namely: EMG amplitude (a) and frequency shift (b) during the maximal fatigue test (black n=16; white n=14), and EMG amplitude (c) and frequency shift (d) during the submaximal fatigue test (black n=16; white n=15).

There was no significant difference between the black and the white group for the percentage increase in force output from the sustained submaximal level with the first electrical muscle stimulation, during the submaximal isometric fatigue test (Figure 4.12). There was also no significant difference between the two groups for the percentage increase in force output with the second muscle stimulation, although this value neared significance ($p=0.06$, Figure 4.12). The difference in the percentage increase in force output between the first and second stimulation was, however, significantly difference between the black and the white group ($p<0.01$, Figure 4.12).

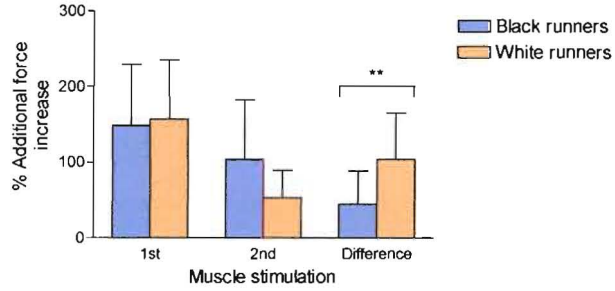


Figure 4.12: The percentage increase in force output from the sustained submaximal level with electrical muscle stimulation during an isometric fatigue test in the black (n=15) and white (n=11) runners. 'Difference' indicates the difference in the percentage increase in force output between the first and second stimulation. *'s indicate a significant difference between black and white groups. **p<0.01

4.5.2.3 Stretch-shortening cycle muscle function

While there was a significant difference between the black and the white runners for the mean force output generated during the push-off (concentric) phase of the SJ ($p<0.05$), there were no significant differences between the groups for the mean force output during the concentric phase of the CJ and the DJ (Figure 4.13 a). There were also no significant differences between the groups for the peak force output generated during the push-off phase of any of the stretch-shortening cycle jumps. As the black and white groups had a significantly different mean body mass (Chapter 2, section 2.5.2.1), the force output during the SSC jumps was also expressed per body mass (Figure 4.13 b). The mean and peak force output per body mass was not significantly different between the black and the white groups for the concentric phase of the SJ, CJ or DJ.

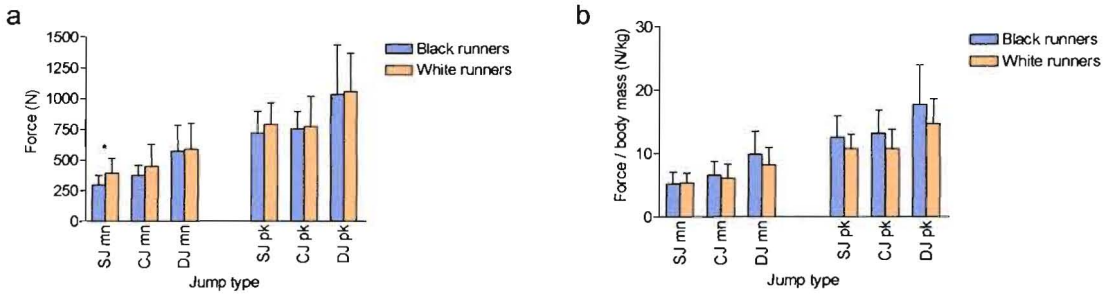


Figure 4.13: Mean (mn) and peak (pk) force output (N, a) and force output per body mass (N/kg, b) during the push-off (concentric) phase of the squat jump (SJ), counter-movement jump (CJ) and drop jump (DJ) in the black (n=16) and white (SJ and CJ n=12; DJ n=13) runners. *'s indicate a significant difference between black and white groups. *p<0.05

There were no significant differences between the black and white runners for EMG amplitude during the concentric or eccentric phases of the SJ, CJ and DJ (Figure 4.14).

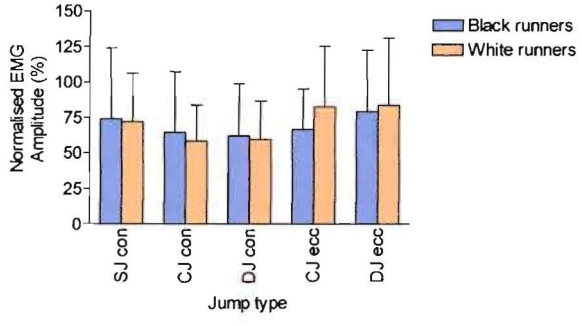


Figure 4.14: EMG amplitude during the concentric (con) and eccentric (ecc) phases of the squat jump (SJ), counter-movement jump (CJ) and drop jump (DJ) in the black (n=16) and white (n=14) runners.

There was no significant difference between the black and the white runners for neuromuscular efficiency (mean force output per normalised EMG amplitude) during the push-off (concentric) phase of any of the three SSC jumps (Figure 4.15 a). There was also no significant difference between groups for neuromuscular efficiency normalised for body mass ((N/kg)/%EMG) during the push-off phase of any of the three SSC jumps (Figure 4.15 b).

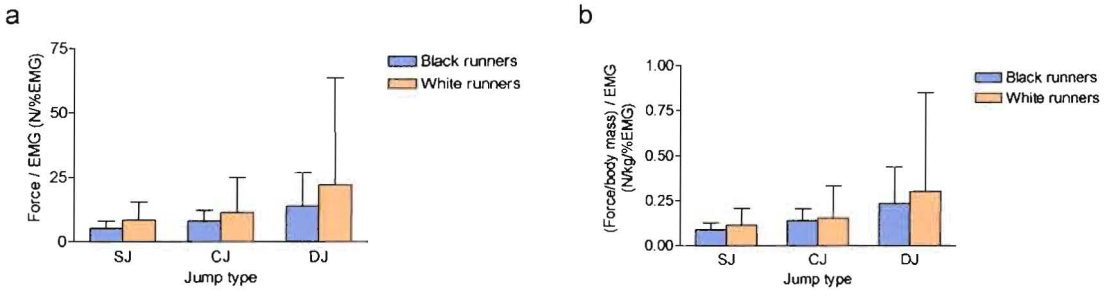


Figure 4.15: Neuromuscular efficiency (mean force output per normalised EMG amplitude (N/%EMG), a) and neuromuscular efficiency normalised for body mass ((N/kg)/%EMG, b) during the push-off (concentric) phase of the squat jump (SJ), counter-movement jump (CJ) and drop jump (DJ) in the black (n=16) and white (SJ and CJ n=11; DJ n=12) runners.

The difference in neuromuscular efficiency between the concentric phase of the three jump types (namely, CJ-SJ, DJ-SJ and DJ-CJ) was calculated to give an indication of the force generated through SSC elastic energy. The differences in neuromuscular

efficiency between the three types of SSC jump are shown for both the black and white runners in Figure 4.16. There was no significant difference between the black and the white runners for either the difference in neuromuscular efficiency (Figure 4.16 a) or the difference in neuromuscular efficiency normalised for body mass (Figure 4.16 b) between the SJ and the CJ, the SJ and the DJ, or the DJ and the CJ.

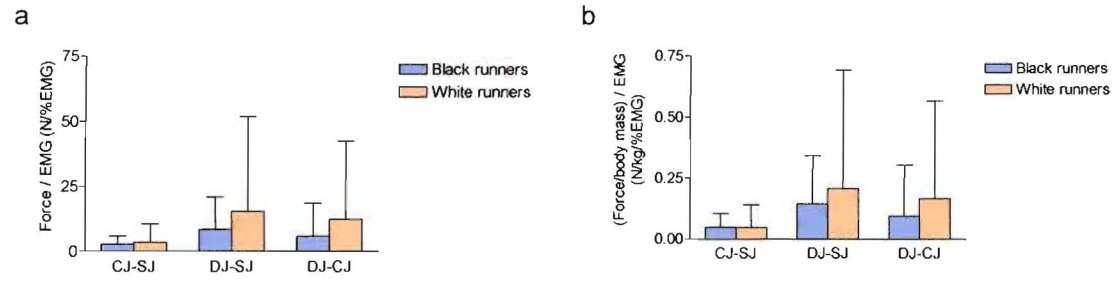


Figure 4.16: Differences in neuromuscular efficiency (mean force output per normalised EMG amplitude (N/%EMG), a) and neuromuscular efficiency normalised for body mass ((N/kg)/%EMG, b) during the push-off (concentric) phase between the squat jump (SJ), counter-movement jump (CJ) and drop jump (DJ) in the black (n=16) and white (SJ and CJ n=11; DJ n=12) runners.

There was no significant difference in the vertical jump height reached by the black and white subjects, with the black runners reaching 41.6 ± 4.31 cm and the white reaching 42.8 ± 6.66 cm.

4.5.2.4 Stride parameters

The mean stride length (when running at 12 km/hr) for the black (2.40 ± 0.09 m) and white (2.40 ± 0.17 m) runners was not significantly different. The ratio of stride length to height however, was significantly different ($p<0.01$), with the black runners covering more distance per stride for their height than the white runners (1.43 ± 0.05 and 1.35 ± 0.09, respectively).

4.6 DISCUSSION

This investigation of neuromuscular factors associated with endurance activity revealed several novel findings with respect to fatigue and endurance performance.

4.6.1 Isometric fatigue

A number of interesting findings related to fatigue resistance during endurance performance resulted from the isometric quadriceps fatigue tests. The first of these was that the runners' time to task failure, often referred to as 'time to fatigue', for the maximal isometric fatigue test correlated significantly with their 10 km personal best time. This suggests that fatigue resistance during static exercise may be related to performance in an endurance event involving dynamic exercise. The runners' 10 km PB was not, however, significantly correlated with TTF for the submaximal fatigue test, suggesting that the relationship between endurance during static exercise and endurance running performance may be dependent on the intensity of the exercise. In addition, the isometric fatigue test only involved contraction of the quadriceps muscles, while running involves many muscle groups, and it is suggested that this relationship be investigated further for other muscle groups used during running.

The second interesting result from the isometric fatigue tests was the novel finding that the black runners' TTF during the submaximal fatiguing contraction was significantly greater than that of the white runners. The black runners' TTF during the maximal isometric fatigue test was also greater than that of the white runners, although the two times were not significantly different. This finding that the black runners could perform for longer than the white runners during static quadriceps exercise suggests that black South African runners have a greater fatigue resistance than white runners, at least during isometric activity. This is similar to the finding of Coetzer et al [84]. In their investigation of elite black and white South African distance runners they found that the TTF during repetitive isometric muscle contractions was longer in the black than the white runners. They found that the black athletes maintained the exercise for 74% of the total time longer than the white athletes, while the black runners studied in this thesis maintained the submaximal isometric contraction for 39% of the total time longer than the white runners. The greater value found in Coetzer's [84] study might have been the result of their protocol involving repetitive contractions while this one involved a continuous contraction, although it could also have resulted from their runners being of elite level, while the ones in this

thesis were of sub-elite level. Nonetheless, the fatigue resistance of the black athletes appears to be greater than that of the white athletes whether repetitive or continuous contractions are performed.

This greater fatigue resistance in black compared to white South African runners has also been shown during running. Weston et al [448], similar to this thesis, tested sub-elite black and white South African runners matched for 10 km race time. They reported a longer TTF for the black runners than the white runners during a submaximal running test, with the black runners lasting 21% of the total time longer than the white. They also concluded that this reflected a greater fatigue resistance in the black athletes compared to the white athletes. The data from this thesis, however, indicates that this superior fatigue resistance is not restricted to running or even to dynamic or repetitive isometric exercise, but occurs during continuous static exercise as well. The submaximal fatigue test result also suggests that the reason black runners generally out-perform white runners in South Africa might not be due, or at least not exclusively due, to size differences between these populations, as size should not affect the isometric fatigue tests.

Inter-individual differences in fatigue resistance during a fatiguing task may be caused by differences in the contractile properties of the skeletal muscle, which are related to the muscle fibre composition, enzyme activities and metabolic system variations [290]. However, there are many neuromuscular factors that can also play a role in inter-individual differences in fatigue resistance, allowing some people to perform better than others. Fatigue-related neuromuscular changes were evident in the rectus femoris during both the maximal and submaximal isometric fatigue tests. The neuromuscular changes, indicated by changes in the EMG parameters, were different for the maximal and submaximal fatigue tests, as would be expected [137].

4.6.1.1 EMG changes during the maximal isometric fatigue test

During the maximal fatigue test, the subjects' quadriceps EMG amplitude, which gives an indication of the level of muscle recruitment in the muscle from which it is measured, decreased significantly with time by 25 %. This finding is consistent with those of previous studies [26,31,228,322,328]. Kay et al [228] reported a 62 % decrease in EMG amplitude during a 100 s isometric maximal voluntary contraction, which is substantially larger than the decrease we observed over a mean of 98 s. The reason for the greater decrease in amplitude in the study by Kay et al [228] is not clear, although it may be related to the subjects participating in the study. While the

subjects tested by Kay et al [228] were recreational athletes, the subjects tested in this thesis were endurance trained, which could have resulted in them showing lesser manifestations of fatigue. Possible differences in fibre type between the subjects in the two studies could also result in the difference [322]. The decline in EMG amplitude with fatigue during this test indicates a reduction in the level of motor unit recruitment, presumably as individual motor units fatigue and stop contracting. The decrease could also reflect the recruitment of proportionally more type 1 relative to type 2 fibres, as type 1 fibres are generally more fatigue-resistant and have smaller action potentials than type 2 fibres [442].

There was a significant compression of, or left shift in, the EMG frequency spectrum during the maximal fatigue test. A left shift in the frequency spectrum, or a decrease in the mean or median EMG frequency, is often used as an indicator of fatigue, and the shift to lower frequencies during a sustained MVC has been shown previously [28,228,321,328]. Kay et al [228] reported a mean percentile frequency shift of 22 % during a 100 s isometric MVC, which is similar to our finding of a 16 % left shift. This left shift in the EMG spectrum with fatigue is presumably the result of a decline in the average motor unit firing frequency and a related decrease in muscle fibre conduction velocity [157,381]. Although not measured, it is probable that the decrease in motor unit firing frequency would be roughly matched to the weakened contractile properties of the rectus femoris muscle, which would optimise force output, in accordance with the theory of muscle wisdom [157].

If there is indeed a decrease in motor unit firing rates during the maximal isometric fatigue test, this could result from a combination of mechanisms. These could involve an altered central drive to the motor units, a reflex inhibition of motor neurons by afferents from the fatigued muscle, and a decrease in facilitatory muscle spindle discharge [157,163,280,456]. The curve indicating the shift in the EMG frequency spectrum in the results is steeper during the first half of the fatiguing maximal contraction than during the second half. This is possibly the result of MFCV decreasing more during the first half of the contraction as a result of the restricted blood flow that results from an intense isometric contraction, while the blood flow would be partially restored during the second half of the contraction as force decreases with fatigue during the trial [460]. It could, however, also result from alterations in the descending central neural command due to changes in pacing strategy during the fatigue test.

4.6.1.2 EMG changes during the submaximal isometric fatigue test

During the submaximal isometric fatigue test, the subjects' quadriceps EMG amplitude increased significantly, in agreement with previous research [328,423,439,446]. This increase in EMG amplitude suggests the recruitment of additional motor units in order to maintain a constant force output as already active motor units fatigue [220,323,423,446]. The augmented motor unit recruitment would compensate for the presumably reduced contractile capability of the fatigued motor units [220]. The increase in EMG amplitude could also reflect the recruitment of motor units with larger action potentials [423], and possibly the recruitment of more type 2 fibres [323], which generally have larger action potentials than type 1 fibres [442]. It would be expected, however, for fatigue-resistant type 1 fibres to predominate during a fatiguing submaximal contraction. Other factors that may also play a role in the observed increase in EMG amplitude are an increased motor unit firing frequency, increased motor unit synchronisation and changes in action potential propagation [423,446].

Although EMG amplitude increased during the submaximal fatigue test, it was not maximal at any stage, reaching 48 % of that produced during an MVC at task failure. Fuglevand et al [151] and West et al [446] found a similar blunted increase in EMG amplitude during sustained submaximal contractions of the first dorsal interosseous and the quadriceps, respectively. This finding indicates that not all of the available motor unit pool was recruited. Therefore, while the increase in EMG amplitude during the trial indicates peripheral fatigue, as additional motor units were recruited to augment the fatiguing ones, the fact that the recruitment never exceeded 50 % of that which is possible suggests that central fatigue might also be occurring. If this inactive 50 % of motor units were not recruited at the endurance limit because they were too fatigued, then central fatigue may not be the cause of the blunted increase in EMG amplitude. But, if these motor units were not recruited because they were being kept inactive as a source of reserve power for emergencies, or because the subjects simply did not wish to tolerate discomfort any longer, then this would indicate the action of central fatigue. It is therefore likely that both peripheral and central fatigue were occurring during the sustained submaximal contraction.

There was a significant left shift in the subjects' EMG frequency spectrum during the submaximal fatigue test. This frequency compression, or shift in the power spectrum to lower frequencies, is consistent with previous reports [290,328,439]. The EMG power spectrum is related to the duration, amplitude and firing frequency of the motor unit

action potentials [137], which in turn are related to the number and type of active motor units [220]. Changes in these factors can therefore lead to a shift in the frequency spectrum. A decrease in the mean MFCV of the active motor units may have resulted in the observed EMG frequency shift [381] by causing an increase in the duration of motor unit action potentials [290]. This decrease in MFCV would probably result from fatigue-related changes in the muscle fibre membrane permeability, which would affect the normal propagation of action potentials [220,233,381]. However, changes in MFCV would probably have been minimal during the submaximal fatigue test as the fatiguing contraction was performed at only 20% of maximal. MFCV is thought to be affected by intramuscular blood flow [290,460], and while during contractions of above 40% of MVC, intramuscular pressure is sufficient to cause ischaemia and therefore affect MFCV, contractions of a lower intensity than this are unlikely to result in a decrease in conduction velocity [460].

The left shift in the EMG frequency spectrum may also have resulted from a decrease in the mean motor unit action potential amplitude, which could in turn have resulted from the progressive recruitment of type 1 fibres, which generally have smaller action potentials than type 2 fibres [442]. In addition, as the frequency content of the EMG signal is also related to motor unit firing rate [220], a decrease in the mean firing frequency of the active motor units could also be causing the observed frequency compression during the fatigue trial. It is perhaps most likely that a combination of these factors resulted in the change in the EMG power spectrum during the sustained submaximal contraction.

The difference in fatigue-induced neuromuscular activity between the maximal and submaximal fatigue tests may be related to the TTF being significantly different between the ethnic groups for the submaximal, but not the maximal test. In accordance with the finding that there was no significant difference between the black and white runners for TTF during the maximal isometric fatigue test, there were also no significant differences between the ethnic populations in neuromuscular changes (as estimated by EMG amplitude and frequency) accompanying fatigue resistance during the maximal fatigue test. There were, however, significant differences between the two groups for the neuromuscular changes with fatigue during the submaximal test. This has not been compared in these two ethnic groups before, and hence these findings are novel.

4.6.1.3 Ethnic differences in neuromuscular activity during the submaximal fatigue test

During the submaximal isometric quadriceps fatigue test the EMG amplitude increased significantly more over time in the white subjects than it did in the black, while the left shift in the EMG frequency spectrum was also greater for the white subjects than for the black. These greater electromyographic changes in the white athletes with increasing fatigue occurred despite them having a shorter TTF than the black athletes during the trial. The greater increase in EMG amplitude for the white runners suggests that there was more recruitment of additional motor units in order to maintain a constant force output for the white than the black runners [220,323,423,446]. This in turn suggests that the reduction in the contractile capability of the quadriceps muscle was greater in the white than the black runners, indicating greater peripheral fatigue in the white runners.

The greater increase in EMG amplitude for the white runners could also reflect more additional recruitment of type 2 fibres by the white than the black runners [323], as type 2 fibres generally have larger action potentials than type 1 fibres [442]. However, the greater left shift in the EMG frequency spectrum for the white athletes would suggest the opposite, that there was a greater progressive recruitment of type 1, rather than type 2, fibres in the white than the black runners. The actual muscle fibre composition of the black and white runners, however, could also be affecting the EMG results. Individuals with muscles made up of a high proportion of type 2 muscle fibres generally have a greater susceptibility to fatigue than do individuals with muscles made up of a high proportion of type 1 muscle fibres [241,434]. Komi and Tesch [241] found that mean power frequency decreased significantly in individuals with a high percentage of type 2 fibres during fatiguing knee extensions, while people with a high proportion of type 1 fibres demonstrated only a slight non-significant decrease. It is therefore possible that the greater TTF exhibited by the black runners, along with the smaller change in their EMG amplitude and frequency, could result from them having a higher percentage of type 1 fibres in their quadriceps muscles than the white runners. However, it was found (Chapter 3, section 3.5.2.1) that there was no significant difference in fibre type composition between the black and white runners studied in this thesis. Similarly, Coetzer et al [84], also reported no difference in percentage fibre type between black and white South African runners, despite finding a difference in their time to fatigue during knee extension exercise.

Assumptions of fibre type activity from examination of the EMG frequency spectrum are generally based on the fact that different fibre types have different diameters; fibres with larger diameters have faster conduction velocities; and conduction velocity affects the frequency content of the EMG signal [130]. However, Farina et al [130] caution that, amongst other problems with this theory, the diameter of type 1 fibres is not always smaller than the diameter of type 2 fibres. EMG amplitude and frequency measurements can also be affected by factors such as electrode position, subcutaneous tissue thickness and composition, and the recording system used [130]. However, in this thesis, the same recording system was used for all EMG measurements for all subjects, and the data was normalised to an MVC to minimise the effect of differences in tissue composition and electrode positioning on subjects. In addition, the EMG data for the isometric fatigue tests indicate changes in EMG activity over time rather than comparing absolute amounts of activity.

Other neuromuscular factors that may be causing the greater change in EMG amplitude and frequency in the white runners compared to the black runners are motor unit firing frequency and MFCV. The greater left shift in the EMG frequency spectrum observed in the white runners could reflect a greater decrease in the mean MFCV of the active motor units [381]. This in turn may result from greater fatigue-related changes in muscle fibre membrane permeability in the white runners than the black runners [220,233,381]. A difference in MFCV between the two groups of runners is more likely to reflect a difference between the groups in their level of peripheral fatigue [395] rather than central fatigue, as MFCV is affected by the metabolic state of the muscle and the intramuscular blood flow [290,460].

Motor unit firing frequency also affects the frequency content of the EMG signal [220], and therefore a greater decrease in the mean firing frequency of the active motor units in the white compared to the black athletes could also be causing the greater left shift in the frequency spectrum observed in the white runners during the fatigue trial. This possible greater decrease in motor unit firing frequency in the white runners could be the result of a greater reduction in central drive to the motor units or a greater reflex inhibition of motor neurons by afferents from the fatigued muscle [163,456]. A greater reduction in the facilitatory effects of muscle spindles on the motor neurons could also result in a greater decrease in motor unit firing frequency [280].

It could be argued that this EMG data is only representative of the rectus femoris muscle, while the maximal and submaximal fatiguing knee extension tests were

performed with the entire quadriceps muscle group. However, Mullany et al [328] found that there was no significant difference in the change in EMG amplitude between the rectus femoris, the vastus medialis and the vastus lateralis during a fatiguing isometric contraction of the knee extensors at 25, 50, 75 or 100 % of MVC. This suggests that the EMG amplitude fatigue pattern is similar for the different muscles in this muscle group. Mullany et al [328] also found, however, that there was a significantly greater percentage frequency compression for the rectus femoris than for the vastus medialis and the vastus lateralis with fatigue. They suggested that this could be due to the rectus femoris showing greater neuromuscular adaptations as a result of either central fatigue or peripheral fatigue.

4.6.1.4 Muscle stimulation reveals both peripheral and central fatigue

The occurrence of central and peripheral fatigue during the submaximal isometric fatigue test was indicated with the use of an electrical muscle stimulation applied to the quadriceps muscle near the beginning of the fatigue test, as well as at the end, before the subjects relaxed. There was a significant difference between the increase in force output caused by the first stimulation and the second, despite the two stimulations being of the same intensity. This difference suggests that peripheral fatigue had occurred during the trial, as the identical external command to the muscle to contract resulted in a reduced force output after fatigue, implying that the contractile ability of the muscle was decreased.

However, the finding that an increase in force output still occurred with the second stimulation suggests that central fatigue was also present. If the muscle were completely peripherally fatigued at this point there would have been no increase in force output. The finding that there was an increase in force output, and therefore reserve capacity in the muscle to contract, yet the subjects chose to end the trial at this point, implies that central fatigue resulted in the decision to stop the test. This is consistent with the findings of Loscher et al [274], who examined fatigue during a sustained submaximal contraction of the triceps surae. They found that when the endurance limit of the voluntary contraction was reached, electrical stimulation of the muscle could generate the same torque level as the voluntary contraction for an additional minute. In addition, the subjects were then able to continue the contraction voluntarily for almost another minute and a half. This finding that voluntary muscle activation was again achievable after one minute of electrical muscle stimulation, which would have involved continued metabolic stress and contractile fatigue

processes, suggests that central fatigue resulted in the initial decision of the subjects to stop the first voluntary contraction.

The percentage difference in stimulated force output between the two electrical stimulations in this thesis did not correlate significantly with TTF during the submaximal fatigue test, implying that the peripheral fatigue measured by this method was not the only factor involved in the length of time the subjects maintained the submaximal contraction. This is also in agreement with central fatigue playing a role in the trial. Therefore, both peripheral and central fatigue appear to be involved during the fatiguing submaximal contraction. Both peripheral and central factors could therefore be related to the different TTF and the different neuromuscular fatigue profiles in the black and white South African runners during the submaximal fatigue test.

The first electrical muscle stimulation to the rectus femoris muscle near the beginning of the submaximal fatigue test increased the force output from the 20% level by a similar amount in the black and the white runners. The second stimulation, however, had a less similar effect on the two ethnic groups. There was a significantly greater reduction in additional force output from the first to the second stimulation in the white runners (104%) compared to the black runners (45%). As the stimulation was externally applied to the muscle, this suggests that the white runners were more peripherally fatigued in their rectus femoris muscle than the black runners were, at their endurance limit. This is despite the finding that the TTF was 76 s longer in the black than the white runners. The better performance, and hence greater fatigue resistance, by the black athletes in the submaximal fatiguing contraction is therefore at least partly the result of a lesser degree of fatigue-related metabolic stress in the muscle, the neuromuscular junction or the spinal reflex loop. This is a novel discovery in the research on the physiological characteristics of black and white South African runners, and is the first finding to narrow the options for the physiological origin of the greater fatigue resistance in the black runners. Collins et al [85], however, described that electrical stimulation over a muscle produces force directly via motor neuron activation and indirectly by reflex spinal motor neuron activation. It is therefore possible that fatigue processes in efferent motor neurons (pre-neuromuscular junction) could also have affected the force output produced by the second muscle stimulation, and therefore central fatigue could also be influencing the greater force drop-off in the white compared to the black runners.

When neuromuscular differences between the two ethnic groups come into play during running, they may be evidenced in the runners' stride parameters.

4.6.2 Stride parameters

Despite the significant difference in height between the black and white runners, their mean stride length when running at 12 km/hr was not significantly different. As a result, the ratio of SL to height was significantly different between groups, with the black runners covering more distance per stride for their height than the white runners. There was a significant negative correlation between SL at 12 km/hr and 10 km PB, suggesting that the longer the strides taken when running at a submaximal speed, the quicker a 10km race is run. This correlation reached a higher level of significance when SL was expressed relative to height, indicating that the further a runner travels with each stride for his height, the faster he runs a 10 km race. Therefore, as SL/height correlates positively with running performance, it is possible that this difference in SL/height between the two ethnic groups could be related to the superior performance of black South African runners compared their white counterparts.

The reason for the difference in SL/height between the two ethnic groups is not known, although it could be related to differences between the groups in the power output of the muscles (due to contractile components of the muscle itself, biomechanical properties or neuromuscular recruitment differences) or to the elastic capacity of the muscles and tendons. However, there was no difference between the groups in the efficiency of stretch-shortening cycle functioning, as will be discussed below, which suggests that this SL/height difference is probably not the result of a difference in elastic energy utilisation. It is therefore more likely to be the result of a greater relative power generation per stride in the black runners, although it could also possibly result from better integration or coordination of the recruitment of the multiple muscles involved in running.

The subjects' mean SL when running at 12 km/hr was 2.40 m, which is slightly higher than the SL value of 2.27 m found by Cavanagh and Kram [75] in male recreational distance runners running at 11.34 km/hr. The SL value for the runners in this thesis is probably slightly higher than that reported by these authors because the runners in this thesis were of a higher performance standard than theirs, as well as the fact that they measured SL at a slightly slower running speed, which results in a lower SL [75].

Distance runners generally choose to run at a stride length that minimises their metabolic cost [76], so it is probable that the SL chosen by the runners was the SL that was most economical to them at that running velocity. If stride length can affect running economy it follows that it may affect running performance. Indeed, the finding that SL and SL/height were significantly associated with 10 km running performance, as described earlier, is in agreement with the report by Brandon and Boileau [54] that SL contributed significantly to 1500 and 3000 m running performance. The finding that SL is related to 10 km running performance has not, to the knowledge of this author, been reported previously. In addition, the difference in SL/height between the two ethnic groups is a novel finding with respect to performance differences in black and white South African runners, and may be related to the greater running economy of the black athletes described in the Cardiorespiratory factors chapter (Chapter 2).

4.6.3 Stretch-shortening cycle muscle function

4.6.3.1 Force output and muscle recruitment during SSC jumps

The jump test results demonstrate the performance-enhancing action of the SSC. The mean vertical force output generated during the push-off (concentric) phase of the CJ was significantly greater than that during the SJ, consistent with previous research [40,48,153,154,178,240]. This augmentation of jump performance with countermovement was probably the result of the storage of elastic energy in the activated muscles during the eccentric phase of the jump, and the subsequent use of this energy in the concentric phase [178], allowing a greater force output during the concentric phase. Reflex potentiation may also be involved, with a stretch-induced increase in muscle spindle activity causing an increase in reflex input to the motor neurons and a subsequent increase in muscle activation [49]. However, examination of the EMG activity during the jump tests suggests that augmentation of jump performance by reflex potentiation is unlikely. The EMG amplitude from the concentric phase of the SJ was significantly greater than that of the CJ, but if reflex potentiation were increasing muscle activation during the concentric phase of the CJ jump then the EMG amplitude should be larger for the CJ and not the SJ.

It is also possible, however, that muscles other than the quadriceps were activated more during the CJ than the SJ. Fukashiro and Komi [153] found that, while the mechanical work of the knee extensors and ankle plantar flexors was similar for a SJ and a CJ, the work by the hip extensors was greater in the CJ. It is therefore possible that a difference in hip extensor activity between the SJ and the CJ could have

influenced the jump performances of the subjects. This demonstrates a limitation to the jump analysis in this thesis. As EMG activity was only measured from the rectus femoris muscle, the activity of other muscle groups, or indeed of the other quadriceps muscles, involved in jumping could not be quantified. The extent of, or the difference in, hip extensor activity during the SSC jumps could therefore not be determined.

The mean force from the push-off phase of the DJ was significantly greater than that from the SJ and the CJ. Komi and Bosco [240] and Viitasalo and Bosco [440] similarly found that subjects jumped higher during a DJ than a SJ, although Häkkinen et al [178] found no difference in jump height between the two. The large concentric force production observed during the DJ is presumably the result of an increased degree of muscle prestretch (stretch during the eccentric contraction) and an increased velocity of prestretch, both of which are associated with enhanced SSC performance [9,48,239,253]. The peak force output during the push-off phase of the SSC jumps showed a similar result to the mean force output, with significantly greater force generated during the DJ than both the SJ and the CJ, but with no difference in force output between the SJ and the CJ.

There were no significant differences between the black and the white runners for the peak vertical force output generated during the push-off phase of any of the SSC jumps. While there were also no significant differences between the groups for the mean vertical force output during the concentric phase of the CJ and the DJ, there was a significant difference between them for the mean force output generated during the concentric phase of the SJ. When the force output during the SSC jumps was expressed per body mass, however, this significant difference between the groups was not evident, such that both the peak and mean force output per body mass were not significantly different between groups for the concentric phase of the SJ, CJ or DJ. This implies that the two groups do not differ in the amount of push-off force they can generate during jumping. This is consistent with the finding that there was also no significant difference in the vertical jump height reached by the black and white runners.

It would perhaps have been preferable to measure the SSC jumps with a protocol that required the subjects to jump up off, and land back on, the forceplate instead of landing off it, as our protocol instructed. This would have allowed the measurement of flight time, which would in turn have allowed the calculation of the height reached by the subjects. However, measuring SSC activity with the subjects jumping forward

off the forceplate is perhaps more specific to running, which involves propelling oneself forward with each step. In addition, the separate measurement of vertical jump height allowed determination of this variable for the subjects, although it could not be related to concurrent force or electromyographic output.

As with the force results, there was no significant difference between the black and white runners for EMG amplitude during either the concentric or eccentric phases of the SSC jumps. The two groups therefore recruited the same percentage of their maximal voluntary recruitment capacity of their rectus femoris muscle when performing the SSC jumps, both during push-off and the preceding stretch. It has been suggested that greater muscle activation with prestretch during the eccentric contraction will allow more attached cross bridges to be stretched and therefore augment SSC performance [9]. As there was no difference in the estimated amount of muscle activity (EMG) during the eccentric phase of the CJ and DJ between groups, this cross bridge related augmentation of performance was presumably not different between groups, at least in the rectus femoris muscle.

4.6.3.2 Neuromuscular efficiency during SSC jumps

Examination of the neuromuscular efficiency of the SSC jumps (the force produced per estimated muscle activity) revealed that the concentric phase of the CJ and the DJ were significantly more efficient than that of the SJ. This indicates that the jumps that used the SSC action (CJ and DJ) were more efficient than the jump that did not (SJ) and therefore that trained distance runners exhibit a typical SSC response during jumping. There was, however, no significant difference in neuromuscular efficiency between the CJ and the DJ, although the comparison neared statistical significance ($p=0.050$).

Despite the runners clearly exhibiting a SSC response during the jump tests, the response might not be as great as that found in most individuals. There was a 20 % increase in concentric force output from the SJ to the CJ for our subjects, while Bosco et al [48] found an increase of 66 % in healthy males. Kubo et al [248] found that long distance runners performed worse in both SJ and CJ, and had a smaller difference in height between the two jump types than untrained individuals. They concluded that this difference in jumping ability could be related to the compliance of the muscle-tendon complex of the vastus lateralis and its potential for energy storage, both of which were lower in the distance runners than the untrained individuals. This may not, however, be a disadvantage for the runners. SSC delta

values for the difference in neuromuscular efficiency between the SJ and the CJ, the SJ and the DJ, and the CJ and the DJ did not correlate significantly with 10 km PB. This means that the runners that benefited more from SSC energy during the jumps did not necessarily have a faster 10 km PB. This suggests that, in trained distance runners, SSC performance during jumping is not associated with 10 km running ability, and that the importance of efficient utilisation of the elastic energy used during jumping may not be of great importance to endurance running performance.

Vertical jump height was also not significantly correlated to 10 km PB, again suggesting that jumping ability is not related to running performance in trained runners. While vertical jump height did not correlate significantly with the difference in neuromuscular efficiency between the SJ and the CJ or the SJ and the DJ, it did positively correlate with the difference in neuromuscular efficiency between the CJ and the DJ. This suggests that the factors that cause a runner to have greater efficiency during a DJ than a CJ are associated with vertical jump performance, although neither of these factors is associated with distance running performance.

Neuromuscular efficiency was not significantly different between the black and the white runners during the push-off phase of any of the SSC jumps. This finding was maintained when neuromuscular efficiency was normalised for body mass. If jump performance had been more efficient in one group than the other it could be proposed that this group used elastic energy more than the other during SSC activity. However, this is not the case and it is therefore likely that elastic energy utilisation is similar between trained black and white South African runners.

SSC delta values for the difference in the neuromuscular efficiency of the concentric phase between the three jump types gives an indication of the SSC elastic energy effect on the jumps. The black and white runners had similar results for both the difference in neuromuscular efficiency and the difference in neuromuscular efficiency normalised for body mass between the SJ and the CJ, the SJ and the DJ, and the DJ and the CJ. The 'difference in efficiency' values are all positive, indicating that the SSC is functioning as would be predicted in both groups, with elastic energy enhancing the efficiency of jumps that involve a prestretch (CJ and DJ). The lack of difference between ethnic groups, however, suggests that neither ethnic group benefited more from the prestretch than the other did. This again suggests that there is no difference in the efficiency of SCC functioning between black and white South African runners (at least during maximal jumping manoeuvres).

It is therefore probable that it is not a difference in elastic energy utilisation that allows black South African runners to perform better than white. This is consistent with the finding that the black group had a longer TTF than the white during the submaximal isometric fatigue test, which did not involve elastic energy utilisation. Together these two findings imply that the superior endurance of black compared to white South African runners is not the result of elastic factors and is not specific to running or even dynamic exercise. Another factor that could potentially be involved in endurance performance and differences in performance between ethnic groups is muscle strength.

4.6.4 Muscle strength

The peak isometric force output, and the peak concentric and eccentric torque output of the quadriceps were not significantly correlated with 10 km PB. This was the case whether the force variables were expressed as absolute values, per body mass or per lean thigh volume, although eccentric torque output per body mass neared significance for the correlation with PB ($p=0.070$). These findings suggest that quadriceps strength is not related to 10 km distance running ability, at least in runners of the standard tested in this thesis. People do not usually produce a truly maximal force output during isometric MVC testing [3]. The 537 N peak quadriceps isometric force output achieved by the subjects in this study therefore reflects their best voluntary effort, rather than the maximal capacity of their muscle for force output. The subjects' peak quadriceps eccentric torque output was greater than their peak concentric torque output, which is generally, but not always, the case for most muscle groups [65,228]. The subjects' isometric and isokinetic strength was only tested for the right leg, however, it has been found that there is no limb strength right/left asymmetry in isometric or isokinetic knee extension in normal men [351].

The quadriceps force output was compared in the two ethnic groups to investigate whether strength differences in muscles used in running could be related to running performance differences between the groups. The peak isometric quadriceps force output was lower in the black than the white runners, as was the peak concentric torque output. The peak eccentric torque output, however, was not significantly different between the groups. This was in agreement with the finding of Coetzer et al [84], that elite black South African runners developed lower peak isometric torques than white runners during a maximal contraction of the knee extensors.

As there was a significant body mass difference between the ethnic groups (Chapter 2, section 2.5.2.1), their force output was also corrected for their body mass. This resulted in there being no significant difference between the groups for peak isometric force output or peak isokinetic torque output. This suggests that the differences in absolute quadriceps strength between the groups may have merely resulted from their difference in body size. However, as the quadriceps muscle group specifically was being examined, the ethnic strength comparison was also conducted with the force output corrected for LTV. This yielded similar results to the absolute, uncorrected force output values, probably because there was no significant difference in LTV between the black and white runners (Chapter 2, section 2.5.2.1). The peak isometric force/LTV and peak concentric torque/LTV were lower in the black runners than the white runners, while there was no difference between the groups for peak eccentric torque output. This finding was again consistent with that of Coetzer et al [84], who found that black elite runners developed lower peak isometric torques than white runners when the torques were expressed per LTV.

Force output can be altered by changing the number of active motor units, the type of muscle fibres recruited and the motor unit firing rate [65,220], therefore any of these factors could be affecting the difference in quadriceps strength between the two ethnic groups. Type 2 muscle fibres are capable of producing a greater force output than type 1 muscle fibres [50,51] and therefore a difference in quadriceps fibre composition between the groups could potentially result in their force output discrepancy. Indeed, Komi and Tesch [241] found that individuals with muscles made up of a high proportion of type 2 muscle fibres demonstrated higher peak knee extension torque than did individuals with muscles made up of a high proportion of type 1 muscle fibres. However, in the Intramuscular factors chapter (Chapter 3), it was found that there was no difference in percentage fibre composition of the vastus lateralis muscle between the black and the white runners.

Perhaps the most interesting of the findings relating to strength was that, while the groups differed in strength isometrically and concentrically, this difference was not evident during the maximal eccentric contraction. The reason for this is not known. However, as neither peak isometric force output nor peak concentric or eccentric torque output correlated significantly with 10 km PB, this finding may not be related to the difference in running performance between black and white runners in South

Africa. However, further work is needed to identify the reasons for this finding and whether or not it could have any relevance to ethnic performance differences.

4.7 CONCLUSION

The findings of this chapter are summarised in Figure 4.17. Performance during the maximal quadriceps isometric fatigue test correlated significantly with 10 km running performance, suggesting that fatigue resistance during static exercise may be related to running ability. The black runners performed significantly better than the white runners during the submaximal isometric fatigue test, suggesting that the previously described superior fatigue resistance of black South African runners is not restricted to running or even dynamic exercise, but occurs during continuous static exercise as well. The EMG amplitude and frequency spectrum changes accompanying fatigue during the submaximal isometric fatigue test suggested less fatigue in the black compared to the white runners. Force output responses to quadriceps electrical muscle stimulation during this trial also indicated less fatigue in the black athletes, and the involvement of both central and peripheral fatigue processes were evident. Both stride length and stride length per height were positively associated with 10 km running ability, and the black runners covered more distance per stride for their height than the white runners, suggesting that this could be related to the superior performance of black compared to white South African runners. The runners demonstrated a normal stretch-shortening cycle response during jumping, however their SSC performance was not related to 10 km running ability. There was no difference in the efficiency of SSC functioning between the black and white runners, and it is therefore probable that it is not a difference in elastic energy utilisation that allows black South African runners to perform better than white. Quadriceps isometric, concentric and eccentric strength were not related to 10 km running performance, and the white runners were isometrically and concentrically stronger than the black runners. In summary, running performance in trained distance runners is associated with endurance during fatiguing static exercise and with stride length, but not with stretch-shortening cycle performance. Black South African runners perform better and show less evidence of neuromuscular fatigue than white South African runners during submaximal static quadriceps exercise. They also cover a greater distance per stride for their height than white runners, but perform no differently during maximal stretch-shortening cycle testing. The superior endurance of black compared to white South African runners may therefore be associated with their superior fatigue resistance and stride characteristics, but is not likely to be the result of elastic factors and is not specific to running or even dynamic exercise.

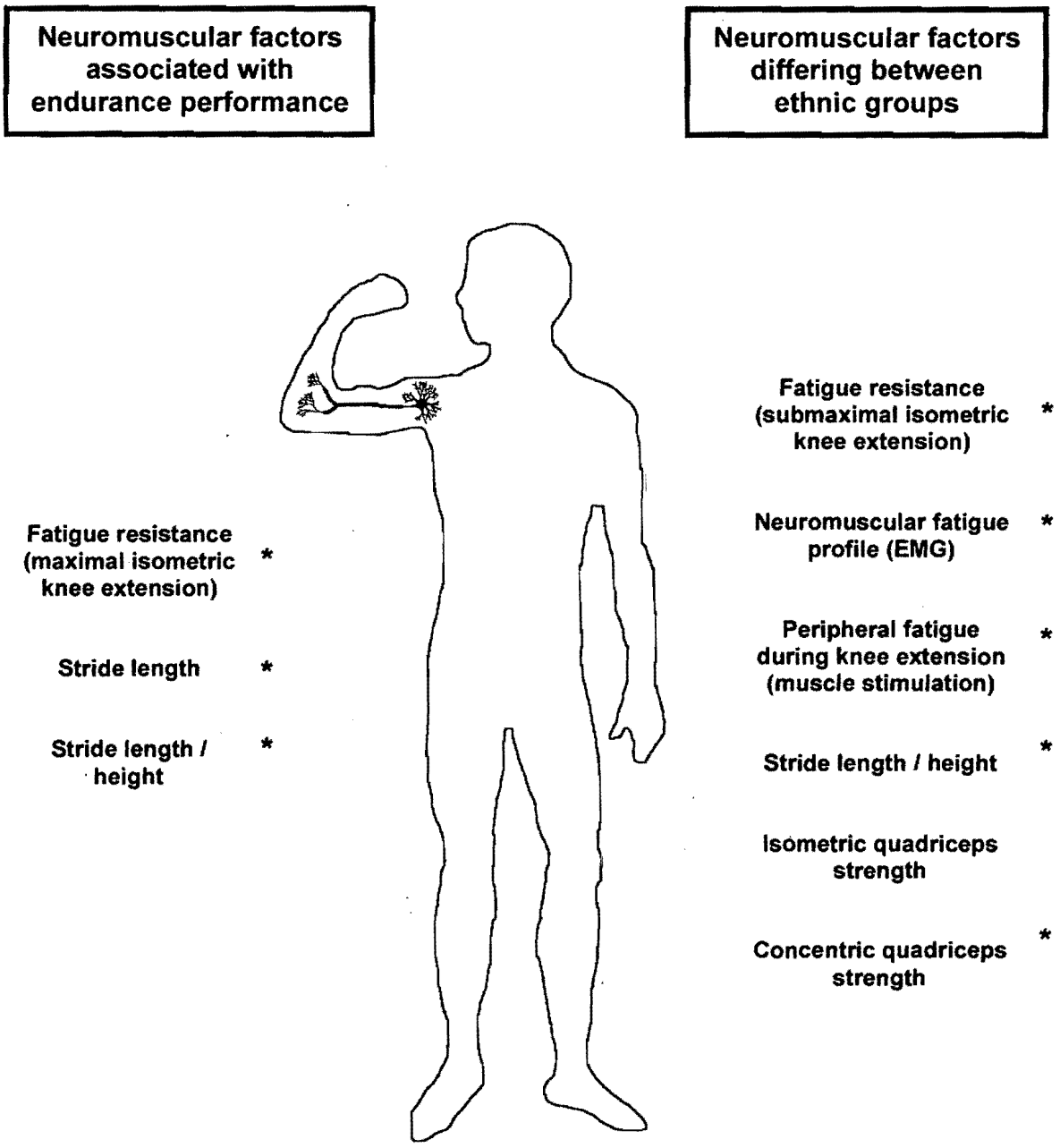


Figure 4.17: Summary of the neuromuscular factors measured in this chapter that are associated with endurance performance and/or are different between black and white South African runners. * indicates novel findings

Chapter 5 Central nervous factors

5.1 PREAMBLE

In the Neuromuscular factors chapter (Chapter 4) it was determined that running performance in trained distance runners is associated with endurance performance during fatiguing static exercise. In addition, black South African runners, who generally outperform their white counterparts in distance races, performed better and showed less evidence of neuromuscular fatigue during sustained static exercise than white South African runners. This suggests that there may be a relationship between endurance performance and neural factors.

Fatigue induced alterations in the neural component of a fatiguing activity include not only changes in motor unit activity, but also changes in efferent motor command, generated in the central nervous system [122]. The involvement of central (neural) factors in fatiguing exercise, described in Chapter 4, is further evident in the reported excessive symptoms of fatigue in patients with chronic fatigue syndrome and patients with 'effort syndromes', which appear to result from central, rather than peripheral mechanisms [265,420]. It has been speculated that physiological factors set the outermost limits to performance, while psychological factors determine the more proximate ones [202]. As psychology is essentially the cognitive representation of brain physiology, this implies that an individual's physical capacity is determined by their physiological limits, while their physical performance is limited by their brain. Indeed, motor drive from the central nervous system can decrease if the individual performing the fatiguing task lacks motivation or does not want to continue to tolerate the increasing sensations of discomfort [27]. Little is known about the neurobiological basis of the sensation of effort during a fatiguing task [122], or how the conscious sensation of fatigue originates [414]. In fact, the central nervous system in general is perhaps the least understood of the systems in the body, particularly with respect to fatigue and physical activity. As such, it has been suggested that the search for factors involved in endurance performance and fatigue should include studies of the central nervous system and psychological factors [338,340,448].

One of the potential methods of examining changes in central nervous system activity with fatigue is to measure changes in the electrical oscillatory activity of the

cortex during a fatiguing exercise using electroencephalography (EEG). It is not yet known how fatigue is related to patterns of electrical activity in different regions of the brain [414], so research in this area should prove informative. Increasing fatigue is also associated with another form of physiological oscillation, that of tremor, the mechanisms of which are also not entirely understood. In general, there is a noticeable lack of study of the functions of oscillations in the motor system, given their widespread nature [294]. In particular, only a very modest amount of study has investigated the relationship between these oscillations and fatigue with endurance performance. This chapter will therefore make use of force fluctuation measurements and electroencephalography to examine fatigue associated tremor and the activity of the brain during fatiguing static exercise.

5.2 LITERATURE REVIEW: The role of central nervous system factors in fatigue and endurance performance

With physical activity, the muscle contraction necessary for movement is controlled by the central nervous system. The central nervous system (CNS) consists of the brain and the spinal cord, and it is activity of the brain that will allow the initiation of voluntary movement as well as the sensations related to exercise, such as fatigue. Indeed, even simply the thought of performing a muscle contraction can lead to physiological changes related to physical activity, such as an increase in heart rate [159]. On the other hand, an athlete may be prevented from using his or her full muscular potential during exercise due to psychological reasons [65]. For example, the degree of fatigue an individual experiences can be affected by motivation as well as the memory of prior physical activity [414].

The earliest indication that a fatiguing task will eventually fail is not the inability to continue producing the necessary force output, but instead a conscious perception that a greater effort is needed to continue the task [122]. Changes occur at all levels of the motor pathway during fatiguing activity [429], so that the fatigue process involves all elements of the motor system, from alterations in the production of neural drive to changes in muscle contractile activity [122]. Some of these fatigue processes involve alterations within the CNS [429]. Fatigue that results from mechanisms proximal to the neuromuscular junction is termed 'central fatigue' [429]. This is evidenced as a "progressive reduction in voluntary activation of muscle during exercise" [157]. Supraspinal fatigue, a subset of central fatigue, is that "produced by failure to generate output from the motor cortex" [157]. Fatigue at the supraspinal level is demonstrated by the fact that transcranial magnetic stimulation (TMS) of the motor cortex during a sustained maximal voluntary contraction can result in additional force output from the contracting muscle [158]. This suggests that there is suboptimal output from the motor cortex, and that central fatigue may include a decreased drive to the motor cortex [429].

It has been suggested that fatigue can include both an increase in the perceived effort required to produce a desired force as well as an eventual inability to produce this force [121]. One of the limits to performance may therefore be sensory tolerance, as the task being undertaken becomes progressively unappealing [157]. As such, any physiological process that contributes to an increased sense of effort can be designated a fatigue mechanism [122]. 'Sense of effort' is often subjectively assessed

using rating of perceived exertion scores, however these do not allow a distinction between the central and peripheral factors that add to fatigue [343]. Neurophysiological measures are therefore needed when attempting to differentiate between central and peripheral fatigue.

5.2.1 The protective effect of fatigue

Enoka and Stuart [122] refer to fatigue as an acute neuromuscular adaptation to sustained activity. It is possible that this adaptation is a beneficial phenomenon, which has a protective effect on the body. The central governor hypothesis, as initially proposed by Hill and colleagues [191,192], modified by Noakes [336,337,340] and further revised by Noakes et al [341], suggests that the human body houses a neural 'governor' that regulates the recruitment of skeletal muscle during exercise in order to prevent the development of a potentially harmful physiological state. Integrating feedback from afferent signals from the body, this governor would cause the termination of exercise, or at least a reduction in exercise intensity, before a deleterious condition, such as anaerobiosis of the heart or brain, could develop.

Ulmer [437] similarly proposed the existence of a feedback control system including a 'programmer', which adjusts muscle power output via efferent motor signals. During closed chain activities, this programmer would take into consideration the finishing point (teleoanticipation). If this governor or programmer indeed exists in the CNS, then this suggests that physiological signals can lead to psychological inhibition of exercise [65]. The investigation of the physiological factors limiting exercise performance should therefore include studies of the central nervous system, which may reveal the possible existence and workings of the proposed central governor [340].

5.2.2 Fatigue signals to the CNS

If the CNS responds to physiological indicators of fatigue, then an important question is: Which fatigue-related peripheral signals does the CNS respond to during exercise? Multiple peripheral afferents may be relaying information to the brain regarding the condition of the muscle contractile structures [74,255]. Group III and IV muscle afferents respond to the local mechanical, thermal and biochemical conditions in the muscle [65,157], and their discharge increases with fatigue [157]. These afferents respond to many different factors, such as changes in lactic acid or potassium ion concentration, which will in turn depend on the length and level of contraction as well as muscle perfusion [99,378]. While these afferents probably act at multiple levels in the nervous system [157], they may particularly exert their effects

'upstream' of the motor cortex [74], suggesting that their influence on motor output is not necessarily direct, and may be associated with cognitive factors in the brain.

Signals from interoceptors and proprioceptors may also influence the CNS [245]. For example, information regarding capillary oxygen levels may be integrated in the brain to prevent ischaemia [340], while muscle spindles act by sensing the amount and rapidity of stretch in the muscle and sending afferent signals to the CNS [65]. In addition thermal receptors may act on the CNS to cause a decrease in neural drive to the muscles, possibly selectively, during exercise in hot conditions [344,379,436]. Similarly, receptors indicating conditions of substrate depletion, such as hypoglycaemia, can also result in reduced neural drive to the muscles during prolonged exercise [343]. When all these factors are considered, is becomes apparent that the CNS response to fatigue probably results from an integration of multiple sensory afferents as well as psychological factors [183].

5.2.3 Brain changes with fatigue

The changes within the brain during fatiguing exercise are also likely to be numerous and varied, and are not well understood. Within the motor cortex there may be changes in the membrane properties of the pyramidal cells due to repetitive activation, changes in the responsiveness of cortical neurons and therefore their input to the pyramidal cells, and changes in excitatory and inhibitory inputs to cortical cells [429]. There may be alterations in the concentrations of neurotransmitters in the brain, and the rest of the CNS, which would affect the efficacy of neural synapses [343,429]. As the brain itself is metabolically active during exercise, the availability of substrates that act as fuels in the brain, such as glycogen, glucose and lactate, may also affect the degree of CNS activation possible [93,124,343]. Indeed, fatiguing exercise has been shown to increase the uptake of glucose and lactate by the brain out of proportion to the increase in oxygen uptake, and this substrate utilisation is even affected by the will, or the intent, to exercise [93]. During physical activity neuronal networks may oscillate with different frequencies [235], such that fatigue may also be associated with changes in the oscillatory electrical activity in the brain [414].

The way in which these changes within the brain are related to the conscious sensation of fatigue is not known [414]. Multiple neural systems in the brain can affect central fatigue [157], and an examination of these systems in relation to the structure and function of the brain is necessary.

5.2.4 Brain structure and fatigue

The brain is made up of the cerebrum, diencephalon, cerebellum, midbrain, pons and medulla [65]. The outer part of the cerebrum is known as the cerebral cortex. The cortex is made up of lobes, which are responsible for many different functions in the human body (Figure 5.1). The frontal lobe, and in particular the prefrontal cortex, is associated with 'higher' functions such as reasoning, planning and decision-making. The posterior part of the frontal lobe contains the motor cortex, which is partly responsible for motor activity [222,371]. The parietal lobe contains the sensory cortex and allows for sensory perception and the integration and interpretation of sensory inputs. The occipital lobe, situated posteriorly in the brain, is responsible for visual perception, while the temporal lobe is associated with auditory perception [159]. The frontal and temporal lobes are also associated with language [222].

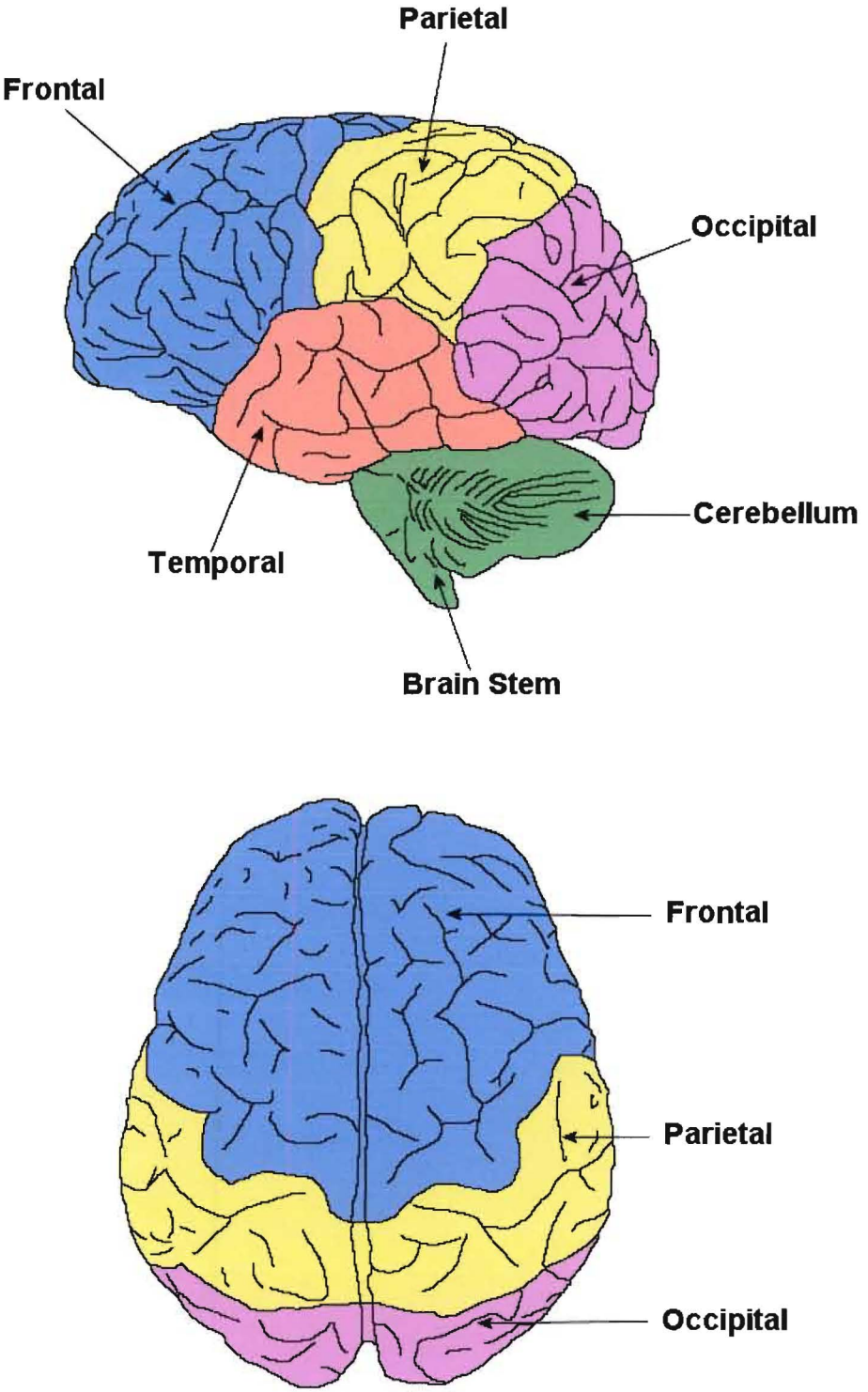


Figure 5.1: Lateral (a) and dorsal (b) views of the human brain, indicating the lobes of the brain.

Fatigue is probably associated with activity of multiple neural ensembles within the brain, with arousal, motivation, attention, tolerance to discomfort, and sensitivity to stress all able to affect voluntary muscle activation [157]. Many different brain regions

may therefore be involved in the generation of the sensation of fatigue, including areas involved in motor control, emotion formation, motivation, memory, decision making, and vocalisation [414]. As such, all of the cortical lobes, as well as subcortical structures and regions within the brain stem and spinal cord, could be involved in endurance performance and the fatigue process. Some of these, particularly cortical, areas and their possible relation to fatigue with exercise will now be discussed. In addition the areas of the brain that may be involved in fatigue are summarised in Table 5.1.

Table 5.1: Summary of the areas of the brain that may be involved in fatigue during endurance activity.

Brain area	Possible function in fatigue
Sensorimotor cortex	Instigation of muscle contraction
	Movement control and planning
	Processing of sensory and motor information
Cerebellum and basal ganglia	Coordination and programming of movement
Visual areas	Sensing and integration of visual inputs with physiological inputs during endurance tasks involving visual guidance
Emotion and memory formation areas	Laying down of memory of fatigue task
	Comparison of fatigue state to memory of previous fatiguing activity
Cingulate and insular cortices	Formation of motivation and behavioural drive
	Integration of sensory and autonomic information
	Detection of deviation from physiological homeostasis
Language areas	Recognition of fatigue
	Self-encouragement during fatigue
Reasoning, planning and decision-making areas (frontal and prefrontal cortex)	Planning of how to deal with fatigue during exercise
	Reasoning whether or not to continue fatiguing exercise
	Decision making of whether or not to continue fatiguing exercise
Thalamus, hypothalamus and reticular formation	Transmission and integration of information from different systems, including sensory and motor, emotions and memory
	Autonomic function and homeostatic regulation
	Control of cortical electrical activity

5.2.4.1 Sensorimotor cortex:

The signal for voluntary muscle contraction stems from the motor cortex, and is transmitted along motor neurons to the muscle fibres [65]. The supplementary and

mesial premotor areas are associated with higher-order movement control and planning [371]. Activity in the cortical motor areas (motor cortex, premotor cortex, supplementary motor cortex) may therefore be linked to central fatigue [429]. The motor and sensory cortices are linked at the central sulcus (where the frontal and parietal cortices meet), and, as the sensory cortex would receive sensory information from the body during exercise, the sensorimotor cortex may be implicated in the fatigue process.

With an increase in force output from a muscle, there appears to be increased activity and excitability in the contralateral primary motor cortex [104]. Similarly, an increase in cerebral activity (using regional cerebral blood flow measurements) has been reported in the leg motor region with bicycle exercise [450] and in the contralateral hand and forearm motor regions during a submaximal handgrip task [450]. This relationship between motor cortical activity and exercise appears to exist even with imagined activity. Using hypnosis, Thornton et al [433] found that subjects displayed more activity in the supplementary motor areas, the right premotor area and the superolateral sensorimotor areas when imagining cycling uphill as opposed to freewheeling downhill. This also suggests that afferent information may not be essential for the generation of a signal of descending motor command or effort [155].

While these studies have indicated motor cortical activity with exercise, it has been suggested that the sense of effort during exercise (and therefore the sensation of fatigue) is linked to the activity of neural centers 'upstream' of the motor cortex, rather than merely being the result of a corollary of the central motor command [74]. Indeed, transcranial magnetic stimulation of the motor cortex during a sustained maximal voluntary contraction can result in additional force output from the contracting muscle, suggesting that there is inadequate neural drive 'upstream' of the motor cortex, resulting in central fatigue [158]. In addition, studies examining brain activity during both static and dynamic exercise have found no change in activity in motor or sensory related structures in the cortex with time, and hence with increasing fatigue [103,345] (in normal temperatures). The contradictory findings of activity or lack of activity in the sensorimotor cortex with exercise may be because the exercise- or fatigue-induced changes in cortical behaviour may be different for different muscles [157]. The contradictory findings may also result from the fact that some of the studies simply examined cortical activity changes with exercise, while others specifically investigated cortical activity changes with fatigue during exercise.

Fatigue appears to result in a depressed excitability of the motor cortex [156,157]. Indeed, the level of intracortical facilitation in the motor cortex (reflecting the excitability of interneuronal circuits in the motor cortex) may be related to the amount of exercise that can be performed as well as the level of fatigue in the motor cortex after exhaustive exercise [430]. As discussed previously, it has been suggested that the motor cortical cells are not the primary site of the generation of central fatigue despite being involved in many of the processes associated with it, and that cells 'upstream' of the motor cortex are particularly important in central fatigue [157]. Therefore, if the motor cortex is not the primary, or only, site of fatigue in the brain, other areas of the brain need to be assessed.

5.2.4.2 Cerebellum and basal ganglia:

Two other areas involved in movement, and therefore possibly fatigue, are the cerebellum and the subcortical basal ganglia [414]. These structures are involved in the coordination and programming of movement, and interact with the motor cortex for the control of movement [159]. Strong basal ganglia activation (as measured by regional cerebral blood flow) has been reported during a sustained submaximal muscle contraction [103].

5.2.4.3 Visual areas:

There are many regions in the brain that are involved in visual perception. The primary and secondary visual cortices, situated in the occipital cortex, function in visual sensing and integration of visual input. Krause et al [245] reported alterations in EEG frequency, which they speculated could be associated with an increase in activation, in occipital leads during fatiguing static muscle contractions. Increased activity (blood flow) has also been found during exercise in brain regions related to vision, including the occipital cortex, in miniature swine [100]. In contrast, Nybo and Nielsen [345] found no significant changes in activity over the occipital cortex with time during dynamic exercise.

The parietal-temporal-occipital association cortex, located where these lobes of the brain meet, integrates visual input with sensory input from the body. As most forms of physical activity involve visual input, this area of the brain may be important in combining the visual information needed to perform the exercise with the bodily sensations formed during the exercise, including those sensations related to fatigue. In a similar manner, visual areas in the parietal and frontal cortices may be related to fatiguing exercise. The parietal area plays a role in the so-called 'visuospatial

sketchpad', which holds and manipulates visual images [13]. The visuospatial sketchpad is part of the functioning of the visual working memory [222], which is a system for the temporary storage and processing of information and acts as a link between sensory information and action [12]. The working memory allows one to remember what one has done recently by means of temporary information storage [222] and so could allow an exercising individual to compare visual cues of their present performance with those remembered from earlier or previous performance. Therefore, as the parietal cortex is associated with multi-modal sensory evaluation [150,371], it may be involved in the integration of multiple fatigue-related inputs, particularly during tasks incorporating visual guidance.

The frontal lobe has also been implicated in the functioning of the visual working memory, in particular the left frontal cortex [422]. This is the location of the so-called 'central executive', assumed to be responsible for the attentional control of working memory [11]. Jonides et al [209] found activation of the right premotor cortex accompanied spatial working memory processes, and Rearick et al [371] argue that, during a visually guided motor task, the visual information received is relayed to the supplementary motor area for integration into the motor command. The visual and motor areas may therefore be active in a linked manner during exercise or fatigue tasks involving visual control.

5.2.4.4 Emotion and memory formation areas:

Emotions and memory are frequently linked, as individuals will often attach an emotional content to a memory, or associate a memory with an emotion they are experiencing. The sensation of fatigue may be associated with unpleasant, or even pleasant, emotions. In addition, during a fatiguing task, an individual may compare their present state of fatigue to the memory of a previous activity, and will probably lay down the memory of the present task for future reference. The areas of the brain related to emotions (e.g. amygdala, ventromedial prefrontal cortex, brainstem nuclei, orbitofrontal cortex) and memory (e.g. hippocampus, parahippocampus, prefrontal cortex) may therefore be important in the generation of the sensation of fatigue [414].

The hippocampus is involved in the conversion of short-term memories into long-term memories [159]. The amygdala, also a subcortical structure and situated close to the hippocampus, is associated with emotions, and may add an emotional content to memories [159]. Unpleasant emotions have been distinguished from neutral and pleasant emotions by activation of the left hippocampus and amygdala [256]. This

suggests that unpleasant emotions associated with fatigue could involve activation of the left hippocampus and amygdala.

If the hippocampus was activated during a stressful task this would probably be associated with activation of the prefrontal cortex as part of the memory formation circuit. Miller [311] described a hippocampo-fronto-parietal system, suggesting that interaction between the hippocampus and the cortex, including the limbic, prefrontal and other areas of the cortex, reflects a basic resonance phenomenon in the brain. The limbic system is associated with emotions [159], and the limbic prefrontal cortex may be involved in the awareness of discomfort during fatigue. Both pleasant and unpleasant emotions can be distinguished from neutral emotions by increased medial prefrontal cortex activity [256]. The orbitofrontal area is part of the limbic cortex and affects the emotional quality of a memory, integrating sensory and cognitive information to interpret the emotional significance of a task and attach that to the memory. Therefore activation of the hippocampus in conjunction with the amygdala and the orbitofrontal cortex may occur during fatigue, as an emotionally appropriate memory of the fatigue is formed.

5.2.4.5 Cingulate and insular cortices:

The cingulate cortex is associated with autonomic and emotional control and plays a role in motivation and behavioural drive [91], factors that would come into play during endurance performance or fatiguing physical activity. The cingulate cortex is activated by pain [91] and has also been described to play a role in error detection [310] and conflict monitoring [52], factors that could be related to unsuccessful efforts to maintain an exercise task with increasing fatigue. While the cingulate cortex may be regarded as limbic motor cortex, the insular cortex may be seen as limbic sensory cortex [91].

The insular cortex is associated with the integration of sensory and autonomic information [390], and has been found to be activated during volitional exercise [451]. Craig [91] proposed a relationship between the left and right, and the posterior and anterior, insula as a way for the brain to detect a deviation from homeostasis in the body. The theory suggests that a mismatch between the activity in the right and left dorsal insula, with greater activity in the right insula, indicates greater sympathetic (relative to parasympathetic) activity in the body, and as such a deviation from homeostasis. This information is then transferred to the right anterior insula, which, along with the right orbitofrontal cortex, is involved in forming subjective emotions,

including those related to exercise stress and pain [91]. Williamson et al [450] found a positive relationship between right insula activation and rating of perceived exertion with exercise. They also found that insula activity increased with increasing intensity of exercise. The insular cortex may therefore be involved in the detection of the deviation from physiological homeostasis generated with fatiguing exercise, and the subsequent formation of the sensation of fatigue.

5.2.4.6 Language areas:

As described by St Clair Gibson et al [414], people 'talk to themselves' when they perform fatiguing exercise as a means of recognising the fatigue and encouraging themselves. These silent 'soliloquies', perhaps taking the form of thoughts such as "I'm getting tired now" or "Keep going!", could possibly be reflected in activity in the language areas of the brain. Broca's and Wernicke's areas of the cortex, situated in the left frontal and left temporal lobe respectively, form part of the speech circuitry in the brain.

Broca's area is involved in the production of language as well as language comprehension [38]. Situated in the inferior frontal gyrus [222], it has been reported to be associated with the subvocal rehearsal system [352], and possibly verbal reasoning [374]. In addition, it may be involved in the imagery of motion [38]. Wernicke's area is situated in the posterior part of the superior temporal gyrus [222] and is involved in recognising speech patterns. Wernicke's area connects with Broca's area via neuronal relays in the cerebral cortex from the temporal lobe to the frontal lobe. If an individual does engage in silent vocalisation or 'self-talk' during fatiguing exercise, it is possible that these language areas of the brain may be active, allowing that individual to acknowledge their fatigue.

5.2.4.7 Reasoning, planning and decision-making areas:

The 'higher' functions of reasoning, planning and decision-making are thought to occur mainly in the frontal and prefrontal cortex. During fatiguing physical activity it is sometimes necessary to plan how to deal with and complete the exercise, to reason whether or not to continue with the exercise if the sensation of fatigue is unpleasant, and to make the decision whether or not to continue exercising. These areas of the brain may therefore be involved in modulating the feelings associated with the sensation of fatigue and planning or deciding how to respond to the fatigue.

Dettmers et al [103] found that activity of the right dorsolateral prefrontal cortex correlated with the duration of exercise during a sustained submaximal contraction, and suggested that this activity may reflect CNS processes involved in over-riding fatigue. The right dorso-lateral prefrontal cortex was also more active in hypnotised subjects imagining they were cycling uphill, compared to when they were imagining freewheeling downhill [433]. Nielsen et al [331] and Nybo and Nielsen [345], however, reported no changes in activity (using electroencephalographic techniques) over the frontal cortex with dynamic exercise. Frontal activity was altered, however, during dynamic exercise in a heated condition. Whether any of these frontal and prefrontal areas of activity relate specifically to reasoning or decision-making is not clear, as there are other functions associated with the frontal and prefrontal cortex, such as emotion generation, as previously discussed.

5.2.4.8 Thalamus, hypothalamus and reticular formation:

The subcortical thalamus has connections to numerous other parts of the brain and relays information between them. As such it is involved many neural systems, including sensory and motor control, emotions and memory [159]. This involvement in the transmission and integration of information from many different systems means that thalamic activity would be relevant to fatiguing exercise in multiple ways. The hypothalamus also has many functions. It is involved in autonomic function and the regulation of many different factors in the body, including temperature and endocrine/neuroendocrine control [159]. It is important for homeostatic regulation, which is critical during fatiguing physical activity.

Both the thalamus and the hypothalamus, therefore, probably play multiple roles during exercise. They may, for example, allow activation of the sympathetic nervous system and transmission of neural information regarding sympathetic nervous activity to relevant parts of the cortex [91,419]. Using hypnosis, Thornton et al [433] found that subjects displayed greater thalamic activity when imagining cycling uphill as opposed to freewheeling downhill, suggesting that thalamic activity is increased even with imagined exercise.

The reticular formation, situated in the medulla and midbrain, is involved in many physiological functions related to exercise, including the regulation of heart rate, blood pressure, respiration and stretch reflexes. The reticular activating system, within the reticular formation, is involved in setting the levels of electrical activity in

the cortex. The relevance of cortical electrical activity to exercise and fatigue will be discussed in the EEG section of the literature review below.

5.2.4.9 Activation of multiple areas:

Clearly there are many regions of the brain that may be involved in the generation or the modulation of the sensation of fatigue during physical activity. Instead of merely originating from activity in any particular one of these areas, fatigue may be an integrative process involving multiple brain regions [414]. These regions may function together by being active simultaneously or may be activated at different times. Chen and Herrmann [77] reported temporal progression in event-related cortical activity, with the activity first in the contralateral somatosensory area, followed by the centro-frontal area and then the centro-parietal area after a painful stimulus. They suggested that this pattern of activity could reflect the transfer of sensory information from perception to cognitive consciousness. The activation of multiple brain areas with exercise has been shown in miniature swine. Measured using regional cerebral blood flow, activity was found to occur in several subcortical areas involved in locomotion control, as well as in brain regions related to cardiorespiratory control and vision [100]. It is probable that multiple brain areas are active in the fatigue process in humans.

5.2.4.10 Hemispheric differences in activity and fatigue:

Exercise may differentially influence the degree of activation in the two cerebral hemispheres [354]. The ratio of right to left hemisphere activity has been found to change after aerobic physical activity [354]. It has been suggested [94,96] that activity in the left and right frontal and prefrontal cortices is representative of the emotional valence of a task, with left hemisphere activity indicating positive, approach-related feelings or behaviour and right activity indicating negative, withdrawal-related behaviour. Based on studies of dispositional anger, an approach-related motivational tendency with negative valence, Harmon-Jones and Allen [184] suggested that anterior asymmetry reflects motivational direction rather than affective valence. This is consistent with reports that disgust and anxiety are associated with right-sided anterior cortical activity [95,97]. This hemispheric differentiation may be present in the anterior temporal cortex as well as the frontal and prefrontal cortices [95,370].

Reduced activation of the right hemisphere in comparison with the left has been suggested to account for the reduction in anxiety associated with exercise [354]. In a

similar manner, increased activation of the right hemisphere may be related to the unpleasant or avoidance-related emotions associated with fatigue.

5.2.5 Electroencephalography (EEG)

Oscillatory activity in the CNS is not well understood, however, interest in this area has increased in recent years [294]. Electrical oscillations may function as a basic form of communication between cortical cell assemblies [235]. They may allow synchronous neural firing between neuronal populations that are functionally related, yet are distributed apart in the brain [287]. Synchronous or coherent activity between different areas in the cortex (or indeed between cortical and subcortical structures, or between brain and muscle) might allow for a temporal link between these different neuronal populations, allowing coordinated functioning [287]. It is not known, however, how fatigue is related to patterns of synchronisation of electrical activity in the brain [414].

Cortical activity can be non-invasively picked up as the electroencephalogram [287]. EEG measures the electrical activity in the brain by recording the variations in potential over the scalp. It can be used to examine both temporal and spatial properties of cortical excitation, however the electrical activity recorded generally originates mainly from the more superficial layers of the cortex [159]. During mental or physical activity different neuronal networks in the brain oscillate with different frequencies [235]. Oscillatory activity at specific frequencies in different brain areas may be related to functional links between these areas. The frequencies of activity in the brain are also affected by physiological changes, such as alterations in blood glucose level, body temperature, arterial partial pressure of CO_2 and hormone levels [159].

The EEG power spectrum is commonly broken down into different frequency bands. Although the defined frequency limits of these bands vary slightly depending on the literature source, they are roughly delineated as: delta (<4 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (13-20 Hz) and gamma (20-40 Hz) [138,159]. The upper limit of the beta frequency band is often extended to 30 Hz, and the gamma band then defined as including frequencies greater than 30 Hz. Since delta band activity is mainly observable during sleep and low-arousal states, it will not be discussed further. As EEG can measure the relative power at these different frequencies over different parts of the brain, and hence detect changes in activity in different parts of the brain, it may be useful in determining which areas of the brain might be associated with fatigue. Indeed, time-frequency analysis of EEG has been shown to be sensitive to

the difference between painful and non-painful stimuli [77], and so may similarly be sensitive to fatigue stimuli. A more detailed discussion of the different frequency bands, and their relationship with exercise and fatigue follows.

5.2.5.1 Theta frequency band:

The theta rhythm has been described as the 'fingerprint' of all limbic structures, and is particularly prominent in the hippocampus [269,270]. Theta-modulated signals are thought to influence limbic, prefrontal and other areas of the cortex in a hippocampo-fronto-parietal system [311]. As discussed earlier in this literature review (section 5.2.4.4), activity in the hippocampus and prefrontal cortex is associated with memory activity in the brain. In agreement with this, theta activity appears to be associated with memory [235], with theta oscillations involved in the transfer of information between the working memory and the long-term memory [391]. Theta activity also increases when performance of a learned task is improving most rapidly, and then declines as the task becomes familiar [311]. Activity in the theta frequency band may therefore play a role in fatigue by an involvement in the learning of fatiguing tasks, the laying down of memories of fatigue sensations during exercise, and the accessing of memories of prior fatigue.

5.2.5.2 Alpha frequency band:

The alpha frequency band is generally the dominant rhythm in awake humans, at rest, with their eyes closed [159]. The band is often broken into an upper and lower frequency band, with these higher and lower frequencies associated with different functions in the brain [236]. Activity in the alpha band is hypothesised to be associated with attentional processing [236,278,402,445]. Klimesch et al [236], however, contend that, while the lower alpha frequencies reflected attentional demands such as alertness and expectancy, the upper alpha band may instead be associated with sensory-semantic processing. The alpha rhythm is marked in the parieto-occipital area [159], and increased alpha activity has been reported in the occipital region in response to visual stimuli [396]. Alpha frequency activity may therefore be important in visual attention. Ray and Cole [370], however, found greater parietal alpha activity for tasks not requiring attention to the environment, such as mental arithmetic, than for those requiring such attention.

It has been suggested that when patches of neurons display coherent activity in the alpha band, an active processing of information is unlikely and that instead the corresponding networks are in a deactivated state [357]. Klimesch [235], however,

suggests that while synchronous activity of large cortical areas may reflect mental inactivity, synchronous oscillatory discharge of selected and comparatively small cortical areas instead reflects mental activity. It is therefore not entirely clear whether synchronous alpha activity in a particular area of the cortex, such as in the visual cortex, would indicate active processing in that area or rather a deactivated state.

A significant increase in the alpha peak frequency has been reported in both occipital and central (over the boundary of the frontal and parietal cortex) leads during fatiguing static muscle contractions [245]. Nielsen et al [331], however, found no change in alpha activity over the frontal areas with dynamic exercise. The different findings in these two studies may be the result of the different areas of the scalp from which the EEG was recorded, or the result of the different types of exercise performed, or simply the result of differences in the techniques of measurement and analysis. More research is therefore needed to identify the alpha frequency response to exercise and fatigue.

5.2.5.3 Beta frequency band:

EEG beta rhythm is often observed over the frontal regions [159]. As described earlier in this literature review (section 5.2.4.7), the frontal lobe is associated with cognitive functions such as reasoning and decision-making, as well as motor activity. Activity in the beta frequency range may therefore be involved in these processes. Brown [67] describes that motor cortical drive of muscle discharge is associated with beta and Piper (30-60 Hz) band activity, with corticomuscular coherence during weak to moderate strength isometric contractions found in the beta band. In agreement with this, Kristeva-Feige et al [246] found corticomuscular synchronisation in the beta frequency band between the contralateral motor cortex and the flexor digitorum superficialis muscle during a submaximal isometric contraction. The contraction was performed with visual feedback of the force level, and the authors concluded that beta range synchronisation is associated with attention directed towards a motor task.

In a comparison of dynamic exercise during the heat and in normal temperatures, it was found that, while beta activity over the frontal areas decreased in the heated condition, the control trial showed no significant changes in beta power over time during the exercise bout [331]. Beta frequency band activity may therefore be involved in motor cortex or 'higher' centre cognitive functioning, suggesting that its link to

fatigue may be via motor activity or through functions such as reasoning and decision-making.

5.2.5.4 Gamma frequency band:

Activity in the gamma frequency band is thought to be involved in muscle contraction and movement [2,67,313-315,365]. In particular, gamma activity is believed to be associated with the control of strong contractions, with the oscillations reflecting both focused attention and the efferent drive to the muscle [314,315]. The role played by gamma activity in movement is not fully understood, but it seems to be related to the execution of both ballistic and sustained movements [2]. It has been suggested that patterns of synchronisation in gamma band activity may facilitate the interaction of the various areas of the cortex involved in movement execution [67]. The role of gamma frequency band activity during fatigue may therefore be of particular importance during intense, rather than weak muscle contractions, as well as for the control of movement with fatigue.

Investigation of EEG activity in the cortex during fatiguing activity will therefore allow insight into which areas of the brain are active or changing in activity with fatigue, as well as which areas of the brain may be functioning together in either the development of, or the resistance against, fatigue. Increasing fatigue is also associated with another form of physiological oscillation, that of tremor. With fatigue during physical activity, an increase in the fluctuation of force output is evident. The physiology of this phenomenon will now be discussed.

5.2.6 Fatigue associated tremor

Tremor may be defined as "a rapid back-and-forth movement of a part of the body" [294]. Physiological muscle tremor involves oscillations across a range of frequencies [294], and the multiple peak frequencies of oscillation during active contraction may allow frequency coding of motor commands [295]. Many sites of origin have been suggested for physiological tremor, including the muscle contractile structure, the motor units, mechanical resonance of body parts, reflex loop activity and CNS oscillations [294]. In addition, these factors may influence each other and may serve to amplify or dampen tremor, even if not the direct source of it [294]. Physiological tremor during non-fatiguing contractions probably originates from central oscillations [295], rather than peripheral feedback loops. However, it is likely that there is involvement

from multiple factors, with different factors causing tremor under different circumstances [294].

Along with an increased sense of effort, fatiguing exercise often results in increased unsteadiness and tremor [157]. This increased tremor with fatigue can be viewed as a form of "enhanced physiological tremor" [294]. This tremor can be detected as the fluctuations in the force output from the contracting muscle during a 'constant' force isometric contraction. The amplitude of the force tremor increases with increasing fatigue during submaximal isometric contractions [116,273,275]. This increase has been found to occur in a non-linear manner, with a steeper increase in tremor amplitude during greater levels of fatigue [273,275]. The frequency of force tremor also changes with fatigue. Tremor mean power frequency decreases with time during fatiguing submaximal isometric contractions [92,273], and the tremor power in individual frequency bands, ranging from 1-50 Hz, has been reported to decrease during a sustained maximal isometric contraction [346]. The nature of the tremor may change with the force of the isometric contraction being maintained, with a stronger contraction resulting in a greater tremor amplitude [5].

A fluctuating force output with fatigue requires greater energy expenditure by the muscle than a smooth force output and results in less accurate task performance [157]. This would be detrimental during physical activity, therefore it is not clear why tremor would increase during fatiguing activity, when this would increase rather than reduce the symptoms of fatigue.

Many sites of origin have been proposed specifically for the fatigue-induced increase in tremor during exercise. Increased tremor with fatiguing exercise may result from changes in the dynamics of muscle contraction, the properties of muscle receptors, proprioceptive reflexes, as well as central factors [157,291]. It has been proposed that muscle tremor in the region of 6-25 Hz probably results from unfused firing of motor units, as they are recruited at about 6-8 Hz and reach total fusion of twitches at about 25-30 Hz [5]. There is less power in the tremor frequency spectrum at the higher frequencies of this 6-25 Hz range as there is greater fusion of twitches in the motor units compared to the lower frequencies. The even lower frequencies are thought to reflect slow force deviations resulting from changes in the net output of the motoneuron pool [5].

Cresswell and Loscher [92] suggested that increasing tremor during a fatiguing submaximal isometric contraction is partially due to altered peripheral afferent input to the α-motor neuron pool. They speculated that the altered peripheral afferent input enhances the excitatory drive to the α-motor neuron pool, increasing the gain of the reflex loop. This augments oscillations in the stretch reflex arc, which results in spurts of motor unit activity and hence tremor. This theory is supported by the finding that continuous tendon vibration or partial ischaemic nerve block, both of which reduce peripheral afferent input from the muscle to the α-motor neuron pool, lessen the increase in tremor with fatigue [92].

In accordance with this hypothesis of bursting activity of the motor units creating force tremor during fatigue, Cresswell and Loscher [92] found bursting activity in the EMG signal at approximately 8-10 Hz during fatiguing isometric contractions. Similarly, the peak frequency of force tremor during a sustained contraction has been found to be at 6-10 Hz [5]. Ebenbichler [116] reported a lower median tremor frequency (10 Hz) during low force level contractions than that during higher force contractions (12 Hz). This is because, at the lower force levels, low-threshold motor units are recruited at relatively low frequencies, and the unfused firing of these low threshold motor units probably results in the frequency content of the tremor [116]. At higher forces, the low threshold motor units will have reached a higher firing frequency and be displaying greater twitch fusion (resulting in less tremor), while higher threshold motor units, with higher twitch fusion levels, are recruited and displaying unfused firing.

Tremor during fatigue may also be increased as a result of increased synchronisation of motor units [294]. Motor unit firing may be synchronised as the result of the units receiving inputs from a common source, or because they are influenced by a common driving oscillation [294]. Changes in tremor with fatigue are also influenced by the recruitment pattern of the motor units during the fatiguing task, namely their timing of recruitment and the motor units' fatigue-related properties [116]. These are in turn related to the type of exercise being performed. As fatigue progresses during a sustained submaximal isometric contraction, there is increased recruitment of high threshold, generally larger, motor units. These larger motor units will have larger action potentials and greater amplitude force twitches [442], which would result in an increase in tremor amplitude. These motor units, however, are likely to fatigue fast [442], and as they fatigue the amplitude of their force twitch would be lowered.

Therefore, during maximal or high force level sustained contractions, when these high threshold motor units are recruited early during the contraction, the tremor amplitude may actually decrease [116].

Therefore, fatiguing exercise often results in increased unsteadiness and tremor. This fluctuating force output increases energy expenditure by the muscle and results in less accurate task performance, which would be detrimental to performance. Although changes in the amplitude and frequency of tremor have been described and many sites of origin proposed for the tremor, these factors are not well understood and hence further investigation is required.

5.2.7 Summary

The central nervous system plays a fundamental role in fatigue during physical activity, partly via the generation of the sensation of fatigue. This sensation of fatigue may function as a protective mechanism for the body during intense exercise. Multiple peripheral signals are relayed to the CNS during fatiguing activity. Changes within the brain during fatiguing exercise are also numerous, although not well understood. In this review, the function of the brain in the context of fatigue has been outlined, and the areas of the brain that may be involved in the generation of the sensation of fatigue were examined. Multiple areas of the brain may be active during fatiguing exercise. The technique of EEG, which measures electrical oscillations emanating from the brain, is a useful tool to investigate central nervous changes associated with fatigue. In particular, the examination of EEG activity in the various frequency bands, namely theta, alpha, beta and gamma, and the cortical locations of their activity during fatiguing exercise, may prove fruitful. Lastly, the relationship of fatigue with another physiological oscillation, tremor, has been discussed, and the causes of fatigue-associated tremor examined. This fatigue-induced tremor, along with changes in oscillatory EEG activity during fatiguing exercise, will therefore be investigated.

5.3 INTRODUCTION

Fatigue is a central component of physical activity and is also one of the symptoms most likely to be reported to medical and psychiatric practitioners [190,289]. Fatigue has been defined in many ways, most describing it as some form of acute physical failure or acute reduction in physical capacity [1,31,46,118]. Gandevia [157] in contrast defined fatigue as "a symptom reported by subjects in whom there may be no obvious defect in muscle performance". More recently, St Clair Gibson et al [414] have suggested that fatigue is a sensation rather than a physical process. They propose that the fatigue that develops during exercise reflects the conscious awareness of changes in subconscious homeostatic control mechanisms, the goal of which is to ensure that exercise always terminates before homeostatic failure occurs [412].

While fatigue is generally classified as either peripheral, usually skeletal muscular, or central, usually brain, the mechanisms of peripheral fatigue have been more widely investigated, especially during exercise. However, many different studies, using methods such as electrical stimulation of the muscle [274,275], hypnosis [202] and pharmacological interventions [202] have shown the presence of central fatigue, which appears to also be the dominant mechanism limiting exercise performed in the heat [344], with hypoglycaemia [343] and in hypoxic or altitude conditions [229].

Indeed, mental excitement can improve voluntary endurance [268] whereas excessive mental 'work' can negatively affect muscular performance [324]. Ikai and Steinhaus [202] showed that increased arousal could offset fatigue, with the observation that force output increased upon firing a gun behind a subject's head. Gandevia [157] described that the behaviour of a muscle is dependent not only on its innate properties, but also on the way in which feedback systems, operating at the level of the spinal motoneuron or at supraspinal levels, control the muscle activity. Fatigue, therefore, has an important central component and the conscious sensation of this fatigue has not been well investigated.

Central nervous system activity has been particularly neglected in exercise physiology, and EEG techniques could be useful in developing knowledge in this area [419]. EEG is a system for measuring regional cortical activity. With sensory or cognitive stimulation, increases or decreases in EEG activity in different frequency bands can be detected. While independence of different frequency bands has not been proved, they are often assumed to have different functions [393].

The majority of studies examining the effect of exercise on EEG activity have focused on alpha band activity [305]. Most studies have investigated the effect of exercise by comparing pre- to post-exercise EEG activity and have found a significant increase in alpha activity after exercise [53,221,354]. Stock et al [419] and Mechau et al [305] reported increased total spectral power directly after both resistance and dynamic exercise across the whole EEG spectrum, while Barthel et al [19] showed a decrease in power in all frequencies after exercise, as well as a shift in spectral power density from higher to lower frequencies. Few studies have examined EEG activity during, as opposed to before or after exercise, due to physical activity causing movement-related artefact in the EEG recordings. Changes in alpha and beta frequency activity have, however, been reported during static exercise and cycle ergometer exercise [245,247].

The effect of fatigue during exercise on cortical activity, rather than the effect of exercise itself, is even less understood, and it has been suggested that EEG be used to investigate brain activity changes that may be associated with the development of the sensation of fatigue [414]. Therefore, in this study we chose to measure EEG activity with the aim of investigating the specific effects of fatigue on cortical activity, rather than the effects of exercise per se. The central theory of fatigue predicts that brain activity should change in an, at least partly, consistent manner in individuals as they fatigue, while a purely peripheral fatigue model would predict that brain activity would not be altered consistently across individuals.

We chose to use the same submaximal isometric fatigue test as that used in Chapter 4 for a number of reasons. Firstly, in Chapter 4, significant differences were found for both the time to fatigue and the neuromuscular recruitment patterns between black and white athletes in this test, suggesting that this test does indeed induce measurable fatigue-related neural activity. Secondly, it was appropriate to use a static, submaximal test to minimise the interference in the EEG signal that can occur with dynamic and maximal exercise. Thirdly, a 20 % of maximal voluntary contraction was used because, although central fatigue occurs during isometric contractions regardless of the initial level of voluntary activation [157], it has been found that low force level sustained contractions produce a greater perception of fatigue than high force level sustained contractions [165,168]. Lastly, Gandevia [157] described how, with 'open loop' exercise in which there is no pre-set duration, the decision to terminate the exercise is voluntary and can thus be influenced by cognitive processes. As it is

these fatigue-related cognitive processes that are to be examined, an open loop fatigue protocol was most appropriate. Electromyography measurements were conducted to indicate the occurrence of typical neuromuscular fatigue profiles, which allows confirmation that the EEG data was measured during a true fatiguing process.

EEG activity was measured during the trial in a group of subjects to determine whether fatigue-associated changes in brain activity were common to multiple individuals. Unlike the previous chapters, runners have not specifically been used as subjects, and black and white athletes have not been compared in this chapter. This study was the first in this laboratory to make use of EEG techniques in the examination of fatigue and it was considered prudent to initially examine fatigue in people in general, before narrowing the investigation to specific athlete groups or ethnic comparisons. The subjects in this chapter include individuals from different ethnic groups (7 black, 11 white, 1 'mixed ancestry'), but no attempt was made to match the groups and ethnic comparisons were not performed. We have suggested [414] that many areas of the brain could be activated to produce the sensation of fatigue. The aim of this study was therefore to investigate which areas of the brain and which frequency bands are activated when fatigue develops during physical exercise.

Along with the electrical cortical oscillations measured by EEG, increasing fatigue is also associated with another form of physiological oscillation, that of tremor [294]. The mechanisms of this form of fatigue-enhanced oscillation are also not entirely understood. The aim of this chapter was therefore to use force fluctuation measurements and electroencephalography to examine fatigue associated tremor and the activity of the brain with fatigue during static exercise.

5.4 METHODS

5.4.1 Subject characteristics

Twenty-five apparently healthy subjects (male and female) from a range of physical activity levels were recruited. Data from six subjects was excluded due to errors or artefact in their EEG recordings. The data from the remaining 19 subjects was analysed. The study was approved by the Ethics and Research Committee of the University of Cape Town Faculty of Health Sciences and all subjects signed informed consent prior to participation in the study.

5.4.2 Experimental design

Subjects visited the laboratory on one occasion. On arrival they signed informed consent (Appendix A, Form 3) and had their height and weight measured. They then performed a maximal voluntary isometric strength test, after which they rested while the EEG equipment was prepared. Baseline resting measures of EEG were then taken, after which a submaximal isometric fatigue test was performed. EEG measurements were recorded during the submaximal trial, and electromyographic (EMG) measurements were recorded during both the maximal and submaximal trials.

5.4.3 Isometric maximal voluntary contraction (MVC) and fatigue trial

The MVC and the fatigue trial both consisted of an isometric knee extension test performed using a Kin-Com Dynamometer (Chattanooga Group, Inc., Chattanooga, USA). The subjects were seated with their hip in 90 degrees flexion and were stabilised in the chair with straps across the chest and waist. They were asked to keep their arms folded across their chest to prevent use of their upper body during leg extension. The tests were performed with the right knee in 60 degrees flexion (0° being the limb in full extension). The subjects warmed-up their quadriceps muscle prior to isometric testing with four 5 s contractions at 50 % of their subjective maximum, two at 70 % of maximum and one at 90 % of maximum capacity.

The MVC required the subjects to perform four maximal voluntary 5 s isometric contractions. This allowed for the determination of each subject's maximal voluntary force output (the contraction with the greatest force value was used), from which 20 % of maximal force output was calculated. The fatigue test required the subjects to perform an isometric contraction at 20 % of maximal force output until task failure. Subjects maintained a steady force output by visually-guided correction of the output (represented as a force trace on a computer monitor) to keep it at the same level as

a target force marker at the 20 % force output level. As EEG was recorded during the fatigue test, subjects were instructed to remain as motionless as possible during the test and to blink as little as possible. A seated, submaximal isometric fatiguing contraction was used in an attempt to minimise the EEG signal interference that occurs with dynamic and maximal exercise.

5.4.4 Force tremor analysis

The fatigue test force data were analysed using MATLAB™ software (The MathWorks Inc., Natick, MA) with additional software courtesy of Dr Hugh Mullany (University College Dublin) and Dr Lance Myers (University of Cape Town) [327]. Tremor amplitude was calculated as the standard deviation of the force during the fatigue trial. The force tremor frequency spectrum was broken down into four frequency bands, namely band 1 (1-3 Hz), band 2 (4-10 Hz), band 3 (11-20 Hz) and band 4 (21-50 Hz), as described by Oda and Kida [346]. Tremor frequency data is given as the power in each frequency band as a percentage of the total power in the signal. The tremor amplitude and frequency data were time normalised. For time normalisation, the data were expressed over ten epochs of equal length between the start and end of the trial, so that the data from all the subjects could be compared, despite different exercise durations.

5.4.5 Electromyographic (EMG) testing

EMG measurements were recorded, processed and analysed as described in Chapter 4, section 4.4.3.

5.4.6 Electroencephalographic (EEG) testing

EEG signals were recorded using a 128 channel Electrical Geodesic™ system, with a sampling frequency of 200 Hz referenced to the vertex electrode. An online bandpass filter from 0.1 to 70 Hz was applied and electrode impedances were kept below 25 kΩ according to manufacturer specifications. Vertical and horizontal eye movements were monitored with a subset of the 128 electrodes. Gasser et al [164] showed that eye movement-related activity (eyes open) in the EEG is found mainly in the subdelta and delta frequency bands (i.e. <4 Hz) and is negligible for frequencies above 8 Hz (i.e. alpha and above). An initial two-minute recording was performed during which the subjects were required not to move and to focus on a single point on a monitor in front of them. This was to obtain a resting baseline level. Subsequent to this, EEG's were recorded during the isometric fatigue trial.

The resultant EEG records were transferred from the Net Station acquisition system for further offline analysis on a personal computer. The outer ring consisting of 18 electrodes was discarded from subsequent analyses due to excessive artefactual recordings. The EEG signals for each electrode and for each subject were processed in the same manner. The EEG data for both the baseline period and fatigue trial were divided into segments comprising 512 data points, or approximately 2.5 s of data, which were assumed to be quasi-stationary. A 512 point Hamming window was applied to each mean subtracted data segment and the fast Fourier transform (FFT) obtained. A power spectrum for each data segment was calculated from the resultant squared magnitude of the FFT of the signal. For the baseline recording, a periodogram was formed by averaging all the power spectral estimates for the recording. The last segment typically had less than 512 samples and was discarded. For the fatigue trial a time normalisation procedure was performed and 10 separate periodogram estimates were obtained for 10 distinct epochs of equal length between the start and end times.

Each periodogram was further divided into 5 relevant frequency bands: theta (4 - 8 Hz), alpha1 (8 - 10.5 Hz), alpha2 (10.5 – 13 Hz), beta (13 - 20 Hz) and gamma (20 - 40 Hz). The definitions of these bands were in accordance with the convention set out in Fisch [138]. The mean power in each band was determined for each periodogram estimate. The band power for each of the 10 time normalised data sets were then further normalised by the baseline, resting band power. This process was conducted because, as expected, there were substantial variations in raw band power across subjects that were significantly reduced by normalisation.

5.4.7 Statistical analysis

Statistical analyses were performed using the Statistica software package (Version 6, Statsoft, Tulsa, OK, USA). The change in EMG and force tremor over time with increasing fatigue (time effect) was analysed using a repeated measures ANOVA. Where significance was identified using the repeated measures ANOVA, the Tukey HSD Post-hoc test was used to identify differences between individual time points. The EEG band power from the normalised data sets in epochs two to ten were compared to the band power from the data set in epoch one. This was to enable the investigation of changes in EEG activity with increasing fatigue rather than with onset of exercise and to eliminate interference due to movement with the onset of contraction. Analyses of changes in EEG over time with increasing fatigue were performed using a repeated measures ANOVA. Post-hoc comparisons between

results obtained at epoch one and at subsequent epochs were performed using a paired t-test with a Bonferroni correction to prevent Type 1 error. Statistical significance was accepted when $p < 0.05$. The force and EMG data for two of the subjects was not accurately obtained due to technical error and was therefore not analysed. This is reflected in the subject number (n) values in the results section of this chapter.

5.5 RESULTS

Of the 19 subjects, 14 were male and 5 were female. The mean age of the subjects was 29 ± 6.9 years, while their mean height and weight were 171 ± 9 cm and 72 ± 15 kg, respectively.

5.5.1 Force output and tremor

The subjects' peak force output during the maximal voluntary isometric contraction was 620 ± 136 N, and their mean time to task failure for the 20 % fatigue test was 188 ± 57 s. The tremor amplitude (standard deviation of the force output) increased significantly over time ($p<0.001$, Figure 5.2).

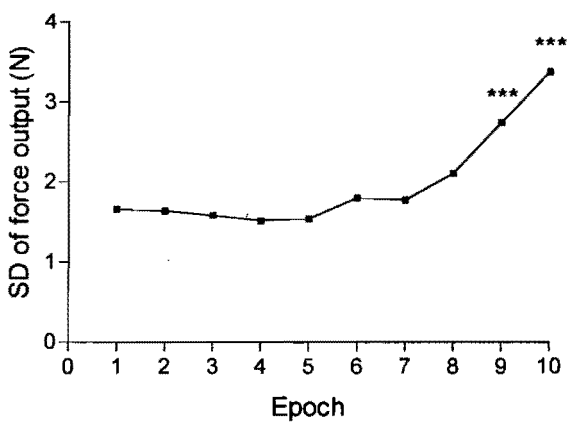


Figure 5.2: Change in force tremor amplitude (standard deviation (SD) of force) during the submaximal fatigue test (n=17). Epoch 1 represents 0-10% of the way through the fatigue trial; epoch 2 represents 10-20% of the way through the fatigue trial, and so forth. *** $p < 0.001$

Of the four tremor frequency bands, there was the most power in band 2 (4-10 Hz), followed by band 1 (1-3 Hz), band 3 (11-20 Hz) and lastly band 4 (21-50 Hz, Figure 5.3). The relative power in band 2 increased significantly over time during the fatiguing contraction ($p<0.001$) and the relative power in band 4 decreased significantly ($p<0.001$). The power in bands 1 and 3 did not change significantly with time.

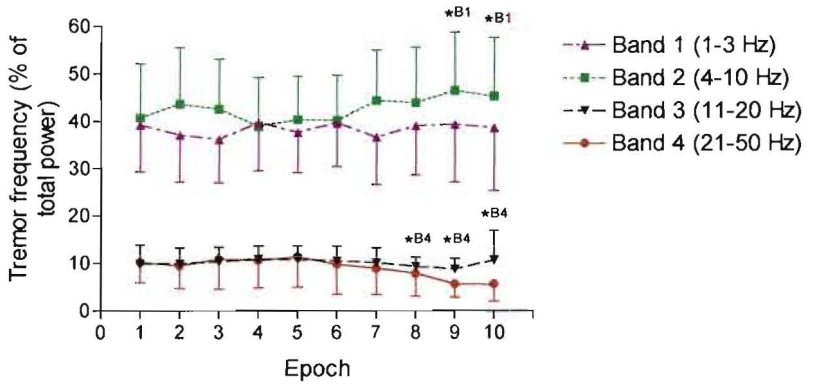


Figure 5.3: Change in force tremor power in four frequency bands during the submaximal fatigue test (n=17). Epoch 1 represents 0-10% of the way through the fatigue trial; epoch 2 represents 10-20% of the way through the fatigue trial, and so forth. * $p < 0.05$ B1: Band 1 B4: Band 4

5.5.2 Electromyographic activity

The subjects' quadriceps EMG amplitude increased significantly with time during the submaximal isometric fatigue test ($p<0.001$, Figure 5.4 a), while their EMG frequency decreased (left shift) significantly with time ($p<0.001$, Figure 5.4 b).

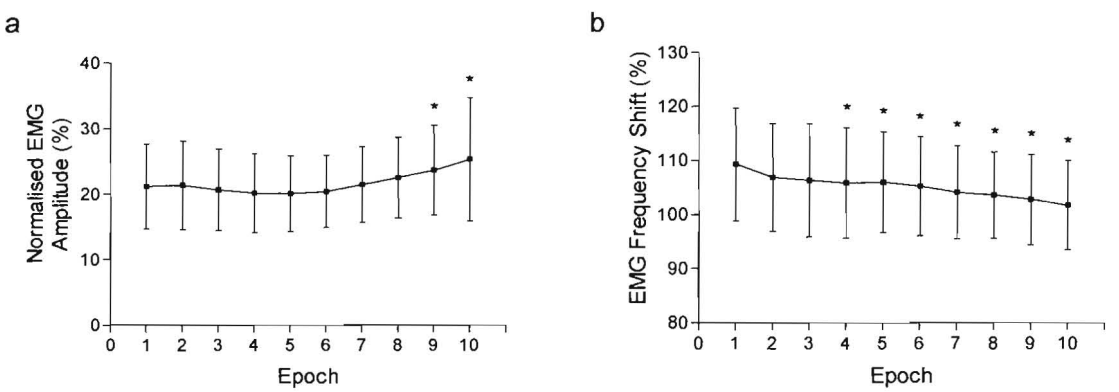


Figure 5.4: Electromyographic amplitude (a) and frequency (b) changes with time during the submaximal quadriceps isometric fatigue test (n=17). *$p<0.05$

5.5.3 Electroencephalographic activity

There were no significant decreases in electroencephalographic activity from the first epoch to any subsequent epochs in any of the frequency bands, over any areas of the brain. The areas of the scalp where significant increases in electroencephalographic activity from the first epoch were observed are shown in

Figure 5.5. As indicated in the figure legend, significant increases in EEG activity from the first epoch are indicated by changes in colour. Dark blue indicates no significant change (for example, in epoch 2 in all frequency bands), while all of the other colours indicate a significant increase in EEG activity. Light blue indicates significance at the $p<0.05$ level and the other colours show increasing levels of significance through to $p<0.0001$, indicated by red.

In order to assist in the interpretation of Figure 5.5, the lobes of the cortex over which the increased EEG activity lies are described in Table 5.2. Epoch one reflects the period 0-10% of the way through the fatigue test; epoch two reflects 10-20% of the way through the test and so forth.

EEG activity increased significantly in the theta frequency band in the seventh, eighth and ninth epochs, with the activity occurring over the occipital and frontal lobes as well as over the border of the frontal and parietal lobes. Activity in the alpha1 frequency band increased significantly from the sixth to the tenth epochs. The activity occurred over the occipital, frontal, and parietal lobes as well as over the borders of the frontal and parietal lobes and the parietal and temporal lobes. Activity in the alpha2 frequency band increased in the fourth and ninth epochs, with the activity occurring over the prefrontal and frontal lobes as well as over the border of the frontal, parietal and temporal lobes. Activity in the beta frequency band was the most altered and was significantly increased from the fourth to the tenth epochs. The activity occurred over all lobes except the occipital lobe, with increased activity over the frontal region predominating. There was no significant change in gamma band activity throughout the trial.

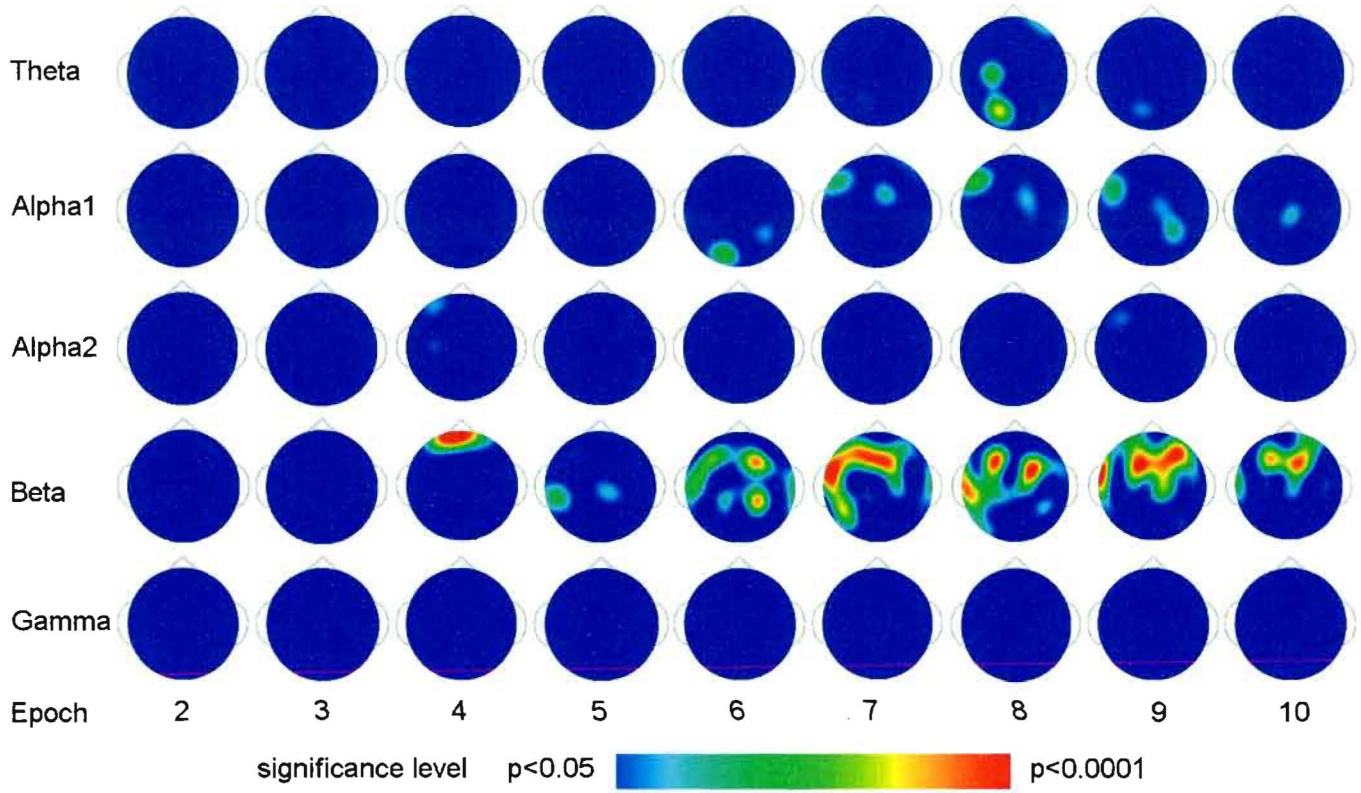


Figure 5.5: Areas of the scalp where there was a significant increase in EEG activity (with the 19 subjects analysed together) compared to epoch 1 for the different frequency bands (n=19). Epoch 2 represents 10-20% of the way through the fatigue trial; epoch 3 represents 20-30% of the way through the trial, and so forth. Each circle represents the head viewed from above with the nose indicated by the triangular shape at the top.

Table 5.2: Areas of the brain over which there was significantly ($p<0.05$) increased EEG activity compared to epoch 1 in the different frequency bands (n=19). Epoch 2 represents 10-20% of the way through the fatigue trial; epoch 3 represents 20-30% of the way through the fatigue trial, and so forth. L: left; R: right; FC: frontal cortex; OC: occipital cortex; PC: parietal cortex; PFC: prefrontal cortex; TC: temporal cortex.

	Epoch								
		2	4	5	6	7	8	9	10
Theta	-	-	-	-	-	L OC	L OC, FC/PC; R PFC	L OC	-
Alpha 1	-	-	-	-	L OC; R PC/TC	L FC; R FC	L FC; R FC	L FC; R FC/PC, PC/TC	R PC
Alpha 2	-	-	L PFC; FC/PC/TC	-	-	-	-	L FC	-
Beta	-	-	L PFC; R PFC	L TC; R PC	L FC, TC, PC; R FC/PFC, PC, TC	L TC, FC, PFC; R FC, PFC, TC	L TC, FC/PFC, FC/PC/TC; R FC, FC/PC, PC/TC	L TC, FC, PFC; R FC, PFC, FC/PC	L TC, FC/PFC; R FC, PFC, FC/PC
Gamma	-	-	-	-	-	-	-	-	-

5.6 DISCUSSION

5.6.1 Electroencephalographic activity

This is the first study to show that there was an increase in EEG activity over all lobes of the cortex, in both cortical hemispheres, and with activity changes in four different frequency bands, during a fatiguing isometric contraction.

Significant changes in the mean EEG activity of the 19 subjects were first observed after 30-40% (epoch 4) of the fatiguing exercise protocol had been completed (Figure 5.6). These initial changes consisted of increases in power in the alpha2 and beta bands. The next frequency band to show significant increases in EEG power was the alpha1 band, with activity commencing after 50-60% of the exercise bout had been completed. The theta band showed significantly increased power from 60-70% of the way through the trial, whereas the gamma frequency band activity did not change. There were no significant decreases in EEG activity in any frequency bands at any epochs.

There were significant increases in EEG activity over many areas of the cortex, which is in agreement with the suggestion that many areas of the brain could be activated to induce the sensation of fatigue [414]. Increased EEG activity was observed over all lobes of the cortex during the fatigue trial in at least one of the frequency bands. Despite the fatigue protocol being a one-legged contraction, there was increased activity over both the right and left hemispheres in the theta, alpha1 and beta frequency bands. Importantly, these areas of activity were significantly increased during the fatigue trial when the data from 19 subjects was analysed together. This novel finding implies that there are changes in brain activity with fatigue that are common to different people. The network of brain areas that are activated with pain is commonly referred to as the 'pain matrix' [406]. It is therefore proposed that, similarly, a 'fatigue matrix' exists in the brain, encompassing the network of brain areas that is activated with fatigue.

Schürmann and Başar [396] have described that cognitive, sensory or motor behaviour could induce multiple frequency band activity at the same time. Most of the increased activity evident in the theta, alpha1, alpha2 and beta frequency bands occurred during the same epochs, with the beta frequency band being active in the most epochs and showing the greatest areas of increased activity. Barthel et al [19] refer to

alpha1 (which they defined as 7-9.5 Hz) as the "known relaxing frequency" and beta1 (12.75-18.5 Hz) as the "activation frequency". As the fatigue trial included physical and mental activity in the form of prolonged fatigue-related stress, it could perhaps be expected that greater increases in EEG activity in the beta than in the alpha1 frequency band would be observed. Nybo and Nielsen [345], however, examined alpha and beta band activity over the frontal and occipital cortex during dynamic exercise, and found no change compared to resting values in the ratio of alpha to beta band activity with time. It is not known, however, how dynamic exercise, such as the cycling trial used by Nybo and Nielsen [345], and static exercise, such as the isometric contraction used in this thesis, might differently affect the relative amounts of alpha and beta band activity in the brain.

5.6.1.1 Beta band activity

The first area of significantly increased activity during the fatigue trial, in the fourth epoch, was over the prefrontal region of the brain in the alpha2 and beta bands (Figure 5.6). The prefrontal cortex is believed to be involved in processing goal-relevant information, and it interconnects with the sensory and motor cortex as well as with limbic structures that process 'internal' information relating to homeostasis [310]. These initial changes in brain activity, common to the 19 subjects, could therefore reflect planning of how to achieve the goal of performing well on the fatigue test, by adjusting homeostatic control circuits to deal with the increasing demands of the task.

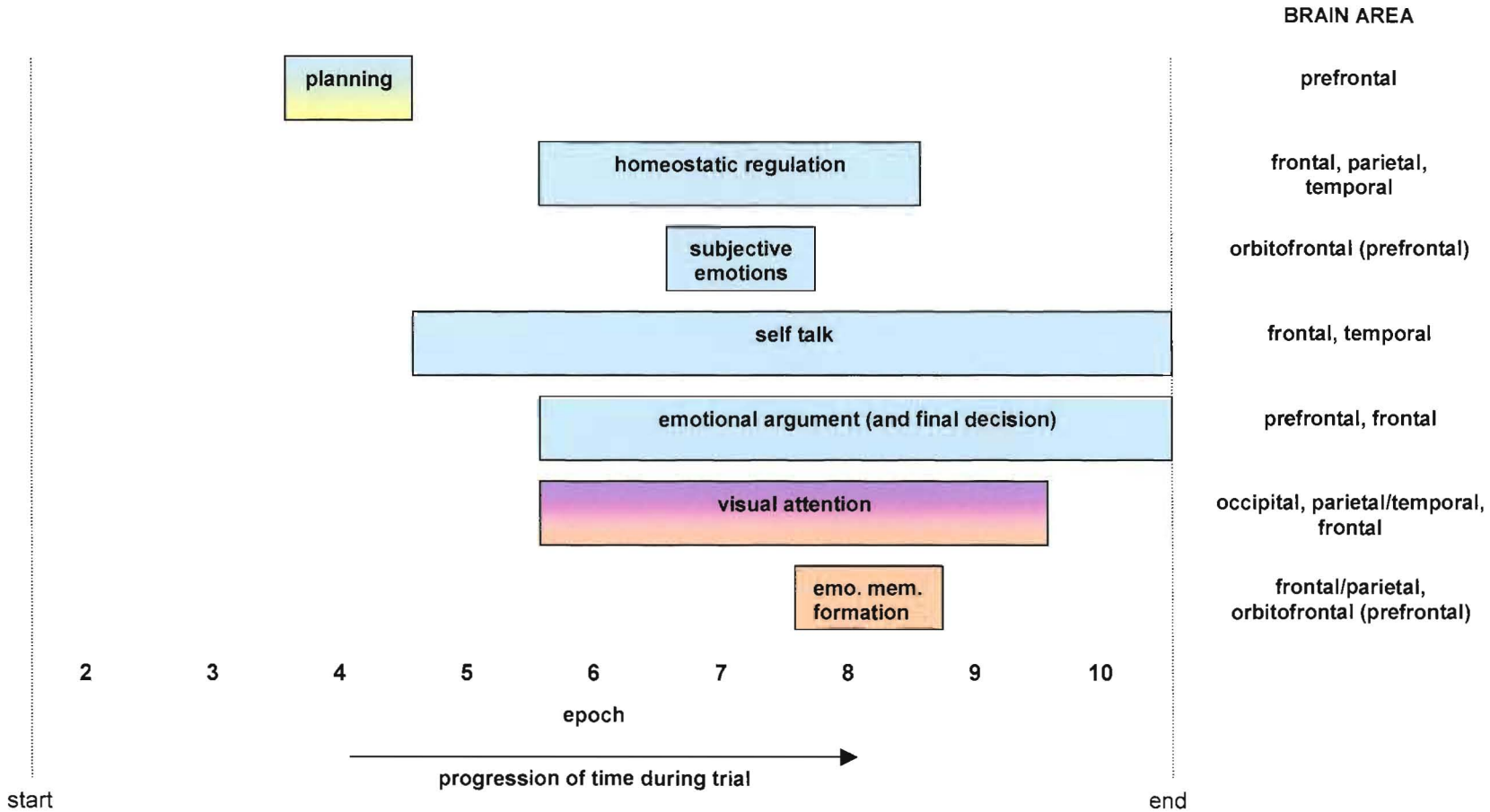


Figure 5.6: Hypothesised functions of brain activity during the fatigue trial. Epoch 2 represents 10-20% of the way through the fatigue trial; epoch 3 represents 20-30% of the way through the trial, and so forth. Emo. mem. formation: emotional memory formation. Beta band activity is blue, alpha1 is purple, alpha2 is yellow and theta is orange.

During the sixth, seventh and eighth epochs, there was increased beta band activity in the frontal, parietal and temporal lobes, and it is speculated that this activity may be related to homeostatic regulation (Figure 5.6). Many regions of the brain are involved in homeostatic regulation and the transmission of afferent signals of homeostatic stress, some of which are deep brain structures such as the hypothalamus, from which activity cannot easily be measured via surface EEG recordings. However cortical regions such as the insula, ventral prefrontal cortex and cingulate gyrus, which are associated with the integration of visceral sensory and autonomic information, may be involved in homeostatic regulation of the internal environment of the body [390]. Craig [91] proposed a relationship between the left and right, and the posterior and anterior, insula as a pathway for the brain to be made aware of a deviation from homeostasis in the body. Williamson et al [450] found that activity in the insula increased with increasing intensity of exercise and also found a positive relationship between right insula activation and rating of perceived exertion with exercise. The insula is surrounded by the frontal, parietal and temporal lobes and it is therefore possible that the activity recorded in this thesis over these areas in the beta band in the sixth, seventh and eighth epochs could reflect activity of the insular cortex. There was also significantly increased beta band activity during the seventh epoch over the orbitofrontal (prefrontal) cortex, which processes information about the emotional valence of a task [91]. This could, along with homeostatic information received from the insula, be involved in forming subjective emotions (Figure 5.6), including those related to exercise stress and pain [91].

In addition, we recently suggested [414] that the language centres of the brain might play an important role in recognising fatigue sensations and initiating conscious changes in behaviour to reduce the activity causing the fatigue. There is increased activity over the left frontal and left temporal regions in the beta band during many epochs (epochs five to ten) and it is in these lobes of the brain that Broca's and Wernicke's areas, the speech areas, are found, respectively. Nikolaev et al [333] found correlations between frontal and left temporal area EEG activity in the beta band when subjects performed tasks requiring word association. If people 'talk to themselves' when they perform fatiguing exercise as a means of recognising the fatigue and encouraging themselves to continue to perform [414], these silent 'soliloquies', could be reflected in beta band activity in Broca's and Wernicke's areas.

During the ninth epoch there was increased activity in the beta band over both the left and the right orbitofrontal cortex, which are part of the prefrontal cortex. Indeed,

in many epochs during the trial the marked frontal region activity that occurred in the beta band extended into the prefrontal area. This activity may be involved in reasoning whether or not to continue with the test. It has been suggested [94,96] that activity in the left and right frontal and prefrontal cortices is representative of the emotional valence of a task, with left hemisphere activity indicating positive, approach-related feelings or behaviour and right activity indicating negative, withdrawal-related behaviour. Harmon-Jones and Allen [184] have suggested that anterior asymmetry reflects motivational direction rather than affective valence. Therefore, the increased activity in the beta band over both the left and right orbitofrontal cortices during the ninth epoch may perhaps indicate the subjects' emotional 'argument' between persevering with the test (approach behaviour) and giving up (withdrawal behaviour, Figure 5.6). Consistent with the theories of Davidson [94,96] and Harmon-Jones and Allen [184], are the findings by Thornton et al [433], who used positron emission tomography to compare two imagined exercise protocols in subjects under hypnosis. When imagining cycling uphill as opposed to freewheeling downhill, subjects displayed increased activity in the right prefrontal and frontal cortices.

During the final epoch, the increased beta activity over the frontal and prefrontal areas was maintained, and may be involved in the final stages of reasoning whether or not to continue contracting. The right prefrontal activity, which is associated with withdrawal behaviour, was substantially greater than the left in this epoch and hence possibly reflects the decision of the subjects to stop the test. This view is supported by the findings of Dettmers et al [103], who reported a correlation between cerebral activity in the right prefrontal cortex and duration of contraction during a fatiguing static finger-press and suggested this could reflect processes involved in over-riding fatigue.

5.6.1.2 Alpha1 band activity

The earliest significant increases in alpha1 frequency band activity during the fatigue trial were during the sixth epoch. This may have coincided with a significant change in attention, as activity in the alpha band has been suggested to be associated with attentional processing [278,402,445], particularly in the lower alpha frequencies [236]. The areas of increased alpha1 activity were over the occipital region and parietal/temporal region (active again in the ninth epoch), which could reflect visual attentional processes (Figure 5.6). As the fatigue task required the subjects to stare at a monitor in order to maintain the correct force output, changes in visual activity

10 Hz [5], and tremor median frequency at 10 Hz [116] during sustained submaximal contractions.

It has been proposed that muscle tremor in the region of 6-25 Hz probably results from unfused firing of motor units, as they are recruited at about 6-8 Hz and reach total fusion of twitches at about 25-30 Hz [5]. The greatest amount of power in band 2 is therefore likely to result from unfused firing of the recruited motor units, especially the motor units that were recruited later during the contraction, which are probably closer to their recruitment threshold and further from total fusion [116]. The decay of power from the lower to the higher frequencies has also been previously reported, and probably results from increasing fusion of the twitch contractions at the higher frequencies [5]. The power at the low frequencies of band 1 may result from slow force deviations resulting from changes in the net output of the motoneuron pool [5].

The relative power in band 2 increased significantly over time during the fatiguing contraction, while the power in band 4 decreased significantly. As the relative power in bands 1 and 3 did not change, this suggests a shift to lower tremor frequencies with increasing fatigue. This is in agreement with previous reports of a decrease in tremor mean power frequency with time during fatiguing submaximal isometric contractions [92,273].

In addition to the theory of unfused motor unit firing discussed earlier, it should be noted that there are other mechanisms that may be resulting in the observed tremor during the submaximal fatiguing contraction. The increasing tremor may result from increased peripheral afferent input to the α-motor neuron pool, which could augment oscillations in the stretch reflex arc, causing spurts of motor unit activity and hence tremor [92]. It may also be the result of increased synchronisation of motor units, changes in the dynamics of muscle contraction, changes in the properties of muscle receptors, or central factors [157,291,294]. Indeed, the increased tremor with fatigue is probably likely to be the result of a combination of these factors.

The relationship between the increasing tremor in this thesis and the EEG signal is not known. It is possible that increasing tremor is one of the afferent signals that the central nervous system uses to interpret the degree of fatigue. As the force tremor is evident in the contracting leg it could be likely that any related changes in the EEG signal would be located in the contralateral sensorimotor cortex. However, as described earlier, this area showed little change in activity during the fatigue task. In

addition, as the force tremor was most evident in the 4-10 Hz frequency band, it could be expected that, if there was synchronisation of cortical and tremor frequencies, the EEG signal would show most tremor-related changes in the theta and alpha1 frequency bands. However, there was no change with fatigue over the leg region of contralateral sensorimotor cortex in either the theta or the alpha1 frequency band.

It is possible that the increased tremor with fatigue is related to changes in activity of the cerebellum or the basal ganglia. However, as these structures are too deep to be accurately measured via EEG, further speculation on this is not possible. Along with the tremor in the 4-10 Hz frequency band, there was also a large amount of tremor in the 1-3 Hz band. This frequency range would correspond to the delta EEG frequency band, which was not measured in this thesis. It is also possible therefore, that the effects of tremor on the EEG signal may have been occurring at these delta frequencies.

5.6.3 Electromyographic activity

The subjects' quadriceps EMG amplitude increased significantly during the submaximal isometric fatigue test, which is in agreement with previous research [328,423,439,446], as well as with the results from the same test in the Neuromuscular factors chapter (Chapter 4, section 4.6.1.2). The neuromuscular activity changes during the fatigue trial that the EMG data reflects will not be discussed in detail here as it has already been covered in the discussion of the Neuromuscular factors chapter. Briefly, the increase in EMG amplitude suggests additional motor unit recruitment in order to maintain a constant force output as already active motor units fatigue [220,323,423,446], and may also reflect changes in the type of motor units being recruited, motor unit firing frequency, motor unit synchronisation and action potential propagation [423,446].

There was a significant left shift in the subjects' EMG frequency spectrum during the submaximal fatigue test. This shift in the power spectrum to lower frequencies is consistent with previous reports [290,328,439], as well as with the results from the same test in the Neuromuscular factors chapter (Chapter 4, section 4.6.1.2). Again, the physiological reasons for this frequency shift were covered in the discussion of Chapter 4, but in brief may include a decrease in the mean muscle fibre conduction

velocity of the active motor units, a decrease in the mean firing frequency of the active motor units, or a change in the fibre type recruitment [220,381,442].

The importance of the observed increase in EMG amplitude and decrease in EMG frequency in this study is that it indicates that a typical, or classical, fatigue profile occurred, which gives confirmation that the EEG data was measured during a true fatiguing process. The decrease in EMG frequency was significant from the fourth epoch, which is the same epoch in which the first significant change in EEG activity occurred. It may be speculated that the initial planning of how to deal with the fatigue in the fourth epoch, as described in the EEG section of this discussion, is related to the change in neuromuscular processing reflected by the decline in the EMG frequency. However, the EEG and EMG changes measured in this chapter have not been linked via techniques such as corticomuscular coherence analysis, and therefore no conclusions can be drawn in this respect. In addition, while the compartmentalising of the data into epochs is useful from a statistical point of view, the time periods are arbitrary and non-representative of continuous physiological processes. Despite this limitation, however, the measurement of electrophysiological variables in this chapter has yielded novel findings, which add to the small amount of existing knowledge of central nervous, specifically brain, activity with fatigue.

5.7 CONCLUSIONS

The findings of this chapter are summarised in Figure 5.7. The novel findings of this chapter are that while gamma frequency band activity was unchanged throughout the fatiguing exercise, there were significant increases in EEG activity in the theta, alpha1, alpha2 and beta bands, which occurred over many brain areas and in both cortical hemispheres, with beta band activity predominating. This finding that brain activity changed significantly with fatigue, when analysed from 19 individuals, is consistent with the central fatigue model. The force tremor amplitude increased significantly in a non-linear manner with fatigue, with a steeper increase from approximately 70 % of the way through the fatigue test. There was more relative power in the lower frequencies of the tremor than the higher, with the most power in the 4-10 Hz frequency band, probably resulting from unfused motor unit firing, along with other factors. EMG amplitude increased and frequency decreased with increasing fatigue, as would be expected during a submaximal, constant force output trial. The relationship between the increasing force tremor, the EMG changes and the EEG signal is not clear, and coherence analysis combining EEG, EMG and force tremor measures is suggested for future studies. The different EEG frequency bands showed different patterns of activity with increasing fatigue and hence would appear to reflect different neural circuits, incorporating many brain regions and both cortical hemispheres. It is therefore likely that fatigue is linked to multiple brain regions and processes, and it is proposed that a 'fatigue matrix' exists in the brain, encompassing the network of brain areas that is activated with fatigue.

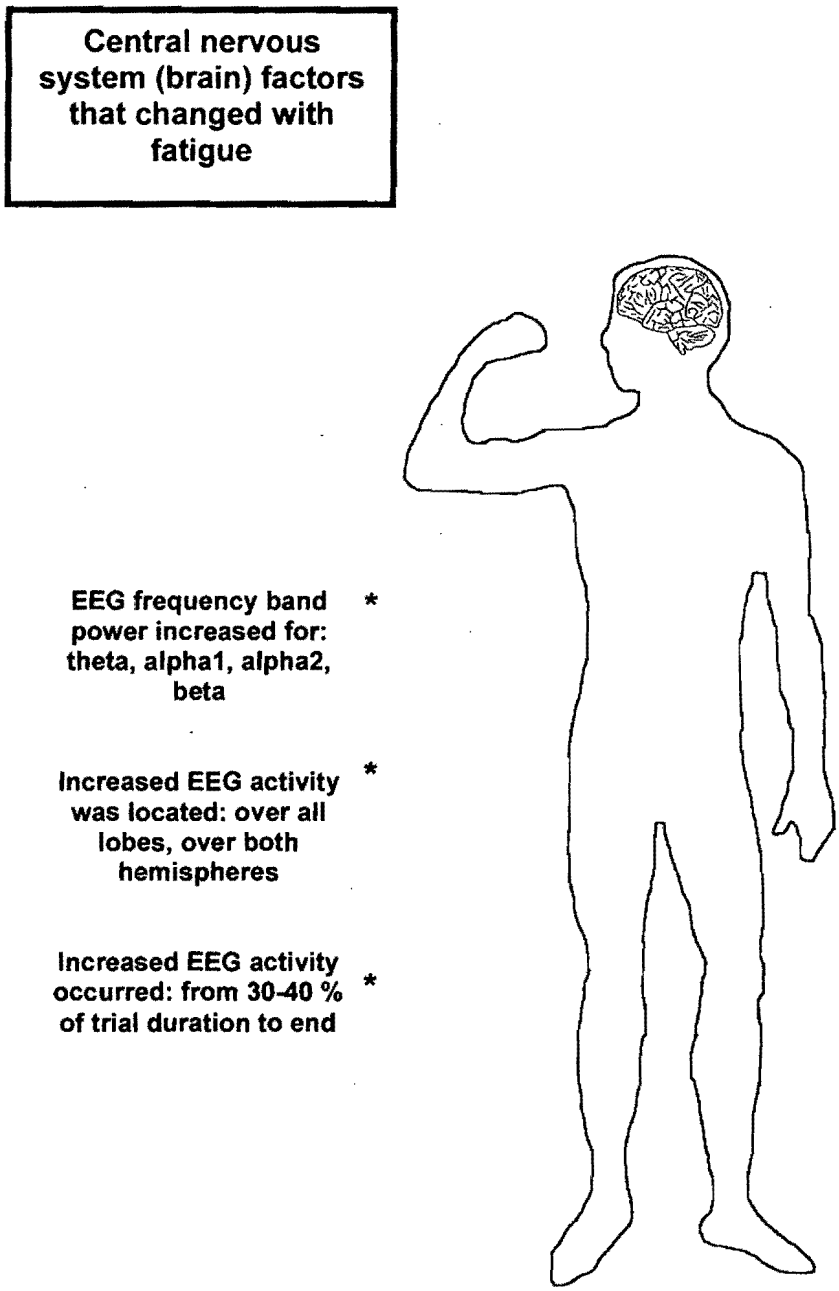


Figure 5.7: Summary of the central nervous system factors measured in this chapter that changed significantly with increasing fatigue during the isometric fatigue trial. * indicates novel findings

Chapter 6 Integration of systems

6.1 PREAMBLE

Chapters 2, 3, 4 and 5 each investigated a different physiological system in the body, namely the cardiorespiratory system, the intramuscular system, the neuromuscular system and the central nervous system, and related specific factors from these systems to fatigue. Each chapter identified physiological factors that were associated with endurance performance. However, despite this reductionist method of examining fatigue being commonly adopted in exercise physiology research, compartmentalising the body makes it difficult to identify the site or sites of fatigue [65]. Indeed, fatigue involves many different physiological factors and processes [65,122,141,255]. The different fatigue processes are interrelated so that they exert interactive effects on each other, and exercising metabolism is probably regulated in an integrated manner [122,255]. Disruption of the integrated functioning of the many different systems, and the elements within them, can therefore also lead to fatigue [65].

It has been suggested that the different physiological factors that may limit endurance performance should be integrated during training and competition [90]. Therefore, an integrated approach is also needed during the research examining these different physiological factors [182]. In this chapter, therefore, the physiological variables from the previous chapters are re-examined in an integrative manner, with the multifaceted nature of fatigue in mind.

6.2 LITERATURE REVIEW: Integration of systems during fatigue and endurance performance

Individual physiological variables cannot account for the complex metabolic behaviour that occurs during physical activity [412]. Similarly, there is no single factor that can account for fatigue or for variations in endurance performance [23,65,255]. Indeed, although many studies have attempted to describe a single factor as the cause of fatigue [122], this "cardinal exercise stopper" has never been identified [157]. Fatigue therefore engages many factors, involving multiple physiological processes and many different mechanisms [65,122,141,255].

The relative importance of these multiple processes and mechanisms is in turn dependent on many factors. Fatigue is task specific, such that the intensity, type and duration of the activity will affect which fatigue mechanisms come into play [65,141]. In addition, the fatigue response to physical activity can be affected by prior experience or antecedent exposures, as well as by the external environment [255]. The different fatigue processes are also interrelated so that they exert interactive effects on each other [122,255]. The various physiological variables may affect each other directly or via indirect feedforward or feedback mechanisms [412]. Therefore, aside from the roles of individual physiological factors on fatigue, the disruption of the integrated functioning of the many different systems, and the elements within them, can lead to fatigue [65].

This leads to the question of what the different interrelated variables and systems are. Essentially, all of the single physiological factors studied separately in exercise and fatigue research are likely to be involved during endurance performance. Muscle biochemistry, neurohumoral factors and central nervous system chemistry are probably all linked during fatiguing exercise [157]. The actual sensation of fatigue probably results from brain activity, which in turn is the result of an integration of sensory afferents from multiple parts of the body as well as psychological factors [183,414]. Along with the psychological and somatic factors, information is probably also integrated from the environment. For example, a runner may sense a change in wind resistance on his skin or visually recognise a change in the gradient of the running surface. The manifold different signalers and feedback systems may operate at different 'speeds' during fatigue, with some activated early or rapidly with exercise, and others later or more

slowly. The purpose of this multiple-structure involvement in fatiguing activity is that physiological homeostasis is maintained, and no single metabolic system progresses to failure during fatiguing activity [255]. Having a large number of different variables involved in feedforward and feedback control could increase the gain of this homeostatic system, making it more robust [255].

The interrelationships between fatigue-related factors in the different physiological systems in the body covered in the Cardiorespiratory, Intramuscular and Neuromuscular factors chapters (Chapters 2, 3 and 4) will now be briefly discussed.

6.2.1 Relationship between cardiorespiratory and intramuscular factors

During exercise the cardiorespiratory system supplies oxygen and substrates to the working muscle via the blood, in order for a high metabolic rate to be maintained [65]. In addition, the circulatory system also removes accumulating metabolites from the exercising muscle, reducing their potential inhibitory actions in the muscle. Therefore, while the skeletal musculature performs the contractile activity necessary for exercise, the cardiorespiratory system balances the supply and removal of substrates and metabolites to and from the contractile tissue (Chapter 2).

Maximal oxygen consumption, or VO_2max, is generally considered to be an index of cardiorespiratory fitness [340], however it is also related to the muscle. Muscle fibre type proportion has been found to be correlated with VO_2max, although this relationship seems to be dependent on the training status of the individuals tested [272]. As described in the Intramuscular factors chapter (Chapter 3), one of the most studied metabolites produced and utilised by the muscle fibres during exercise is lactate. However, lactate is also oxidised in cardiac muscle [63], and the connection lactate forms between skeletal and cardiac muscle, and other organs, allows it to function in metabolic communication between these tissues [179]. One of the major lactate transporters found in skeletal muscle are the monocarboxylate transporters (MCT's). Their role in sarcolemmal lactate transport was described in detail in the Intramuscular factors chapter (Chapter 3). However, MCT's are also found in the heart and blood cells [161], and are therefore also involved in the transport of lactate in the cardiorespiratory system. Lactate and MCT's

therefore form one of the many metabolic links between the skeletal muscle and the cardiorespiratory system.

The energy pathways used in the muscle during exercise, as well as the fibre types recruited, can be affected by the blood flow distribution as well as the circulating levels of blood metabolites, such as lactate, sodium and potassium [66]. The circulating levels of these substances are also partly determined by their concentrations within the specific muscle fibres. For example, there is greater lactate production in Type 2 than Type 1 muscle fibres as a result of higher levels of glycogenolysis, and blood lactate concentration has been found to correlate positively with the percentage of Type 2 fibres during maximal activity [309]. With exercise there is an influx of sodium into muscle cells, while there is an efflux of potassium as a result of the generation and conduction of action potentials. This results in an increased plasma K^+ concentration, the extent of which will depend on the intensity of the muscular work and the activity of the sarcolemmal Na^+/K^+ pump. Increased interstitial K^+ concentrations in contracting skeletal muscle stimulate Group III and IV afferents, which stimulate heart rate and ventilation rate, resulting in increased cardiac output and respiration [263]. It has been suggested that local blood flow is regulated by the accumulation of K^+ in that region [219], and peak blood K^+ concentration has been found to correlate significantly with maximum pulmonary ventilation [316].

Skeletal muscle factors involved in exercise and fatigue are therefore related to the cardiorespiratory system in many ways, and respiratory variables such as oxygen, circulatory variables such as blood flow and metabolite concentrations, and tissue variables, such as transporter protein content in the heart and skeletal muscle all provide connections between the two physiological systems.

6.2.2 Relationship between cardiorespiratory and neuromuscular factors

In the same way that the cardiorespiratory system supplies the muscle with oxygen and substrates and removes accumulating metabolites, it also perfuses the nerves that innervate the muscles. During exercise, the recruitment of motor units is therefore affected not only by the force and speed of contraction, but also the availability of

oxygen and blood-borne substrates [323]. The availability of these substrates as well as the removal of products of metabolism could affect motor unit recruitment during fatigue.

During fatiguing exercise, an accumulation of metabolic byproducts such as lactic acid can reduce intracellular pH and decrease the excitability of the muscle fibre membrane [290]. Changes in the concentrations of potassium in the muscle and blood are also important, as the gradient of potentials across a muscle fibre membrane affects the conduction of excitation along the muscle fibre [290]. A decrease in muscle fibre conduction velocity during a fatiguing contraction can therefore reflect an accumulation of metabolic byproducts [56,264,290]. Muscle fibre conduction velocity has been shown to decrease significantly during a fatiguing static contraction, but not during a fatiguing dynamic contraction [290]. This difference between static and dynamic contractions was probably a result of muscle blood flow being better maintained by enhanced venous return from the contracting muscle during the dynamic contraction than the static, which would result in different metabolic states in these two types of contraction, which would in turn affect the muscle fibre conduction velocity differently.

Despite contraction-induced extracellular K^+ accumulation leading to a change in the gradient of potentials across the muscle fibre membrane, West et al [446] found that the increase in plasma K^+ concentration during a fatiguing contraction was not accompanied by a loss of muscle membrane excitability. They suggested that the decrease in force output may instead be due to K^+ exerting its effect distal to surface membrane action potential propagation, most likely in the T-tubular region. Interestingly, the decrease in muscle force output that occurs with an increased extracellular K^+ concentration has been shown to be negated by addition of lactic acid [332], suggesting a novel potential relationship between these metabolites during fatigue.

Group III and IV muscle afferents respond to the local mechanical and metabolic conditions in the muscle [65,157]. They increase in activity with fatigue as a result of many different factors, such as changes in lactic acid or potassium ion concentration [99,378]. While this relationship initially appears only to involve neuromuscular and muscle metabolic factors, the level of muscle perfusion will affect the concentrations of these metabolites, and hence cardiorespiratory factors will also play a role in this afferent response to fatigue.

6.2.3 Relationship between cardiorespiratory and central nervous system factors

Vissing [441] described an 'exercise reflex', in which the initiation of physical activity generates neural command signals associated with activity of the somatomotor and cerebral autonomic centres, that simultaneously modulate the circulatory, neuro-endocrine and neuromuscular systems. This would allow a coordinated physiological response to exercise. A coordinated response to fatigue would also be necessary to maintain relative homeostasis during fatiguing exercise, and the involvement of the central nervous system (CNS) is integral to this.

Metabolic variables can act as 'sensors' of physiological change, activating afferent feedback to the CNS [255]. The relevance of these metabolites or substrates was mentioned in the previous section (6.2.2) in relation to muscle. However, these variables can also act as 'sensors' via their concentrations in the circulation. For example, fatigue may be affected by changes in the level of blood glucose [79] via afferent signals to the CNS indicating blood glucose concentration [255]. Similarly, plasma interleukin-6 levels, which increase during exercise, may also act as a fatigue signaler to the CNS, as they have a direct afferent input to the brain [409]. These metabolic intermediates can therefore act as signaling agents from multiple areas of the body, aiding in the regulation of physiological systems in an interactive manner [255].

Similarly to the afferent signaling to the CNS concerning the concentrations of metabolites in the blood, information regarding capillary oxygen levels may be integrated in the brain to prevent ischaemia [340]. Interestingly, lactate may be involved in enhancing the afferent neural stimulation of pain sensations during cardiac ischaemia by increasing the sensitivity of ion channels on sensory neurons innervating the heart [203]. This indicates how changes in the levels of metabolites can serve a protective function in the body, as the sensation of pain notifies the individual that there is an imbalance in their body's homeostatic state. The recognition of the pain, or other unpleasant sensation, by the CNS allows the individual to respond in a manner that may help to correct the imbalance. For example, during intense exercise, the sensation of fatigue notifies an individual that it might be wise to reduce their exercise intensity or they may potentially harm their body.

In addition to the brain receiving information regarding the exercising levels of metabolites from other areas of the anatomy, changes will be occurring in substrate levels within the brain itself during physical activity. As well as being oxidised in the skeletal muscle and the heart, lactate is also used as a fuel by the brain during exercise [93,201]. The lactate used by the brain may be produced by the muscle and supplied by the circulation [201,372]. The circulatory system therefore redistributes substances produced during exercise, thereby minimising fatigue by supplying these metabolites as a fuel source elsewhere in the body. The cardiorespiratory system thereby provides the brain with fuel during exercise, and also serves as a link between the muscle and the central nervous system.

6.2.4 Relationship between intramuscular and neuromuscular factors

Muscle metabolic factors and impairment of muscle activation both appear to play a role in human muscle fatigue [312], however the two are inextricably linked. Firstly, the energy requirements of a specific muscle during exercise are dependent on how much that muscle is recruited during the activity [177]. In addition, during exercise, changes in the force output of a muscle can be affected by changing the number of motor units recruited and by changing the firing frequency, and the recruitment strategy used will have an effect on the contractile activity and metabolic processes in the muscle fibres [177]. For example, recruiting a larger number of muscle fibres for a particular exercise intensity would reduce the 'strain' on the excitation/contraction processes in each muscle fibre that is active.

Secondly, many of the physiological and biochemical properties of human skeletal muscle fibres are thought to be determined by the motor nerves that innervate them, and in particular by the firing frequency of the nerve [117,356,392]. In turn, fibre type activity is reflected in electromyographic (EMG) measurements of neuromuscular recruitment. Type 2 fibres generally produce action potentials with greater amplitude and faster depolarisation and repolarisation than type 1 fibres, and therefore have higher conduction velocities [442]. During fatiguing exercise, changes in muscle fibre conduction velocity often occur, and can indicate changes in the muscle fibre recruitment pattern, such as a shift in recruitment from type 2 to type 1 fibres [395]. The relative recruitment of

type 1 and 2 fibres is also reflected in the EMG amplitude and frequencies recorded from the muscle. It has been found that the extent of the change in EMG amplitude and frequency changes with a fatiguing maximal contraction are related to the fibre type composition of the contracting muscle [322].

Thirdly, aside from the relationship between neuromuscular recruitment and fibre type, recruitment is also related to the levels of various metabolites in the working muscle. As described previously, changes in the concentrations of muscle metabolites such as lactate and potassium ions with fatigue can affect the muscle membrane excitability and also stimulate afferents that result in a decrease in recruitment of the muscle [290]. The intra- and extracellular concentrations of these metabolites would depend on the degree of their production and breakdown, as well as their transport across the sarcolemma. The concentration and activity of relevant transport proteins would therefore also play an indirect role in neuromuscular recruitment during fatiguing exercise. For example, the sarcolemmal MCT density will affect the muscle and plasma lactate levels during exercise and therefore also the pH of the muscle cell [62,377], which will result in the stimulation of neuromuscular afferents and possible alterations in neuromuscular recruitment.

The functions of muscle metabolism and neural activity in the muscle are therefore linked in many interconnected ways. The energy used by a muscle fibre during fatiguing activity will depend on how many and which fibres are recruited, and the recruitment of fibres will depend on their size and type, which will in turn depend on their motor innervation. Once recruited, their metabolic activity with the increasing duration of the exercise will initiate a feedback response to again adjust their level of activity.

6.2.5 Relationship between intramuscular and central nervous system factors

Feedback mechanisms from the working muscle can affect neural drive, thereby integrating peripheral fatigue processes with central fatigue processes [177]. Mechanoreceptors and metaboreceptors in the skeletal muscle respond to physiological changes in the muscle with fatigue, such as increases in the concentrations of certain ions or metabolic intermediates. The concentrations of various metabolites may

therefore act as signalers to a central integrating structure, rather than as actual metabolic determinants of absolute fatigue themselves [255].

These are likely to be numerous metabolic signalers in the muscle, such as the levels of glucose, muscle glycogen, interleukin-6, lactate, K^+, H^+ and Ca^{2+} [255,290,409]. These signalers may serve a protective function [336] by providing the CNS with information necessary for determining how the exercise intensity being performed is affecting the homeostatic state of the body. For example, Lindinger et al [263] suggest that fatigue resulting from the loss of K^+ from the muscle cells with exercise serves as a 'safety mechanism', which protects the muscle from the potentially negative effects of continuing to contract in the face of metabolic insufficiency. Afferent signals to the CNS from the muscle may also transmit information regarding the energy status of the muscle, to prevent the exercising body from running out of fuel stores. For example, there may be chemoreceptors in the muscle that are activated in response to low muscle glycogen levels and send feedback to the CNS, possibly via Group III and IV afferents [444].

Muscle lactate levels may also stimulate afferent signals to the CNS [255]. However, as mentioned previously, the production and utilisation of lactate occurs not only in muscle, but in multiple tissues, so that lactate forms a connection between these different tissues. Lactate is therefore involved in whole body metabolism and metabolic communication between tissues [179], and serves as an important substrate involved in energy metabolism in the heart, skeletal muscle and brain [366]. Pellerin et al [353] have described an astrocyte-neuron lactate shuttle, suggesting that MCT isoforms located in the brain function in the transport of lactate from astrocytes to neurons, where lactate acts as an efficient energy substrate during intense activity, allowing the maintenance of synaptic transmission. The various metabolites that fluctuate in the body during exercise can therefore serve as signalers of the local metabolic state in tissues such as the skeletal muscle to the CNS, or act directly within the CNS itself. The information 'transmitted' by these metabolites increases the ability of the CNS to determine the extent of the deviation from resting homeostasis, and hence the progress of fatigue, in the body during physical activity.

6.2.6 Relationship between neuromuscular and central nervous system factors

Efferent signals from the CNS can modulate the level of metabolic activity both directly via neurometabolic pathways, or indirectly via altering muscle motor unit recruitment, which would subsequently affect metabolism in the muscle and other tissues [255,437]. The altered activity of the recruited muscles will lead to mechanical, metabolic and temperature changes in the muscle, which are likely to escalate with the exercise intensity and with fatigue. Group III and IV muscle afferents respond to the local mechanical, thermal and biochemical conditions in the muscle [65,157]. They are activated with muscle contraction [65] and their discharge increases with fatigue [157]. These afferents are likely to exert their effects on the neuromuscular system in multiple ways, including presynaptic, spinal and supraspinal actions [157]. The afferent signals reaching the CNS will be integrated with other somatic and psychological information and result in an increase or decrease in drive from the motor cortex. The change in motor command will depend on the type of fatiguing activity, and will be reflected in a resultant increase or decrease in motor unit recruitment.

The afferent signals that are integrated in the CNS to result in a decreased motor drive do not necessarily act by inhibiting the motor cortex directly. It has been suggested that fatigue causes decreased neural drive upstream of the motor cortex [158], and similarly that the sense of effort during exercise is linked to the activity of neural centers upstream of the motor cortex, rather than merely being the result of a corollary of the central motor command [74]. The peripheral afferent information may therefore be transmitted to various regions of the brain for integration into the formation of a sensation of fatigue and an alteration in motor unit recruitment.

Perceived effort and motor drive are linked. The perception of effort appears to increase exponentially with muscle force [18], which could suggest a nonlinear transformation between the excitatory drive to the motor neuron pool and the sense of effort [122]. Fatigue, however, alters the relationship between the motor command to a contracting muscle and the sense of effort experienced by the subject, presumably as a result of afferent signals regarding the condition of the peripheral contractile structures [74]. The activity of the central nervous system and the neuromuscular system is therefore

intricately linked, and this relationship is affected by fatiguing activity, even as it is involved in the generation of the sensation of fatigue.

6.2.7 Summary

Fatigue during endurance activity appears to engage many factors, involving multiple physiological processes and many different mechanisms. Cardiorespiratory, intramuscular, neuromuscular and central nervous system elements may all play a role in the fatigue process. The relative importance of these multiple processes is dependent on many factors, including the intensity, type and duration of the activity as well as prior experience, the external environment and the physiology of the individual in question.

The different fatigue processes are integrated and affect on each other continuously [122,255]. Fatigue during exercise involves both descending signals from the CNS to the periphery, as well as ascending signals to the CNS from the periphery. As well as having local fatigue effects, tissue-specific and circulating metabolic variables can act as signalers of physiological change, stimulating feedback from the tissues or the circulation to the central nervous system. While the metabolic and neural variables from the different physiological systems all interact with each other, the information from them may be centrally integrated.

It is likely that the purpose of this multiple-structure involvement in fatiguing activity is that physiological homeostasis is maintained, so that no single metabolic system progresses to failure during fatiguing activity. Failure of any physiological system would be hazardous to the body, and therefore an integrative, multisystem regulation of fatigue serves a protective role during physical activity.

6.3 INTRODUCTION

It has been suggested that fatigue involves many physiological factors and processes [65,141,255], and that research is needed to identify the roles and relative importance of these mechanisms in human performance [122]. The different factors involved in fatigue are also interrelated so that they exert interactive effects on each other [255,412], and it has been suggested that research is conducted to examine the interactions between the different fatigue mechanisms [122]. With respect to this thesis, these interactive effects may be within the individual systems described in Chapters 2 to 5, as well as between them. The main findings regarding the factors from the different physiological systems (Chapters 2 to 5) that were associated with endurance performance or that were different between the black and white South African runners is summarised in Figure 6.1.

Therefore, with the assumption that multiple physiological variables are involved in the fatigue process in an integrative manner, the following questions become relevant: (1) Which factors, from which physiological systems are involved?; (2) How do these factors interact with each other?; (3) How is the physiological information that these factors represent integrated?; (4) How does this information result in a change in the physical activity level?; and (5) What is the physiological reason for fatigue? Clearly these questions cannot all be answered in this thesis, however this chapter will highlight how the data from the previous chapters can be examined in a more integrative manner. The

The statistical simple linear regression procedures used in Chapters 2, 3 and 4 were useful in investigating if an independent variable could predict running performance to a certain degree or which, of a few independent variables, was the best predictor of performance. However, if many different variables affect endurance performance, it is important to determine how all of these variables together predict performance, or how well they explain variations in performance. Multiple regression analysis can be used to determine this for a linear system by predicting the value of a dependent variable from a set of independent predictor variables. Multiple regression analysis yields a regression model, or an equation, that expresses the dependent variable as a combination of the predictor variables:

$$y = a + b_1x_1 + b_2x_2 + \ldots + b_nx_n$$

where y is the dependent (outcome) variable, a is the intercept, b_1 and b_2 etc are the regression coefficients, and x_1 and x_2 etc are the independent (predictor) variables. The regression coefficient indicates the estimated increase in the outcome variable for unit increases in the predictor variable. The R^2 value (multiple correlation coefficient) for the equation indicates how well the model fits the data, or how well the model predicts the dependent variable. This statistical method can therefore be useful in determining how a number of physiological variables together predict performance. In this chapter, physiological variables from the previous chapters of this thesis were analysed together in an attempt to shed light on how these different physiological systems can all affect endurance performance and fatigue resistance together.

A series of multiple regression analyses were therefore performed integrating variables from the Cardiorespiratory, Intramuscular and Neuromuscular factors chapters (Chapters 2, 3 and 4). As this thesis is examining endurance performance, 10 km race personal best time (PB) was used as the outcome variable to be modeled. Variables from the Central nervous system factors chapter (Chapter 5) were not included as a different set of subjects were tested in this chapter, many of whom were not runners.

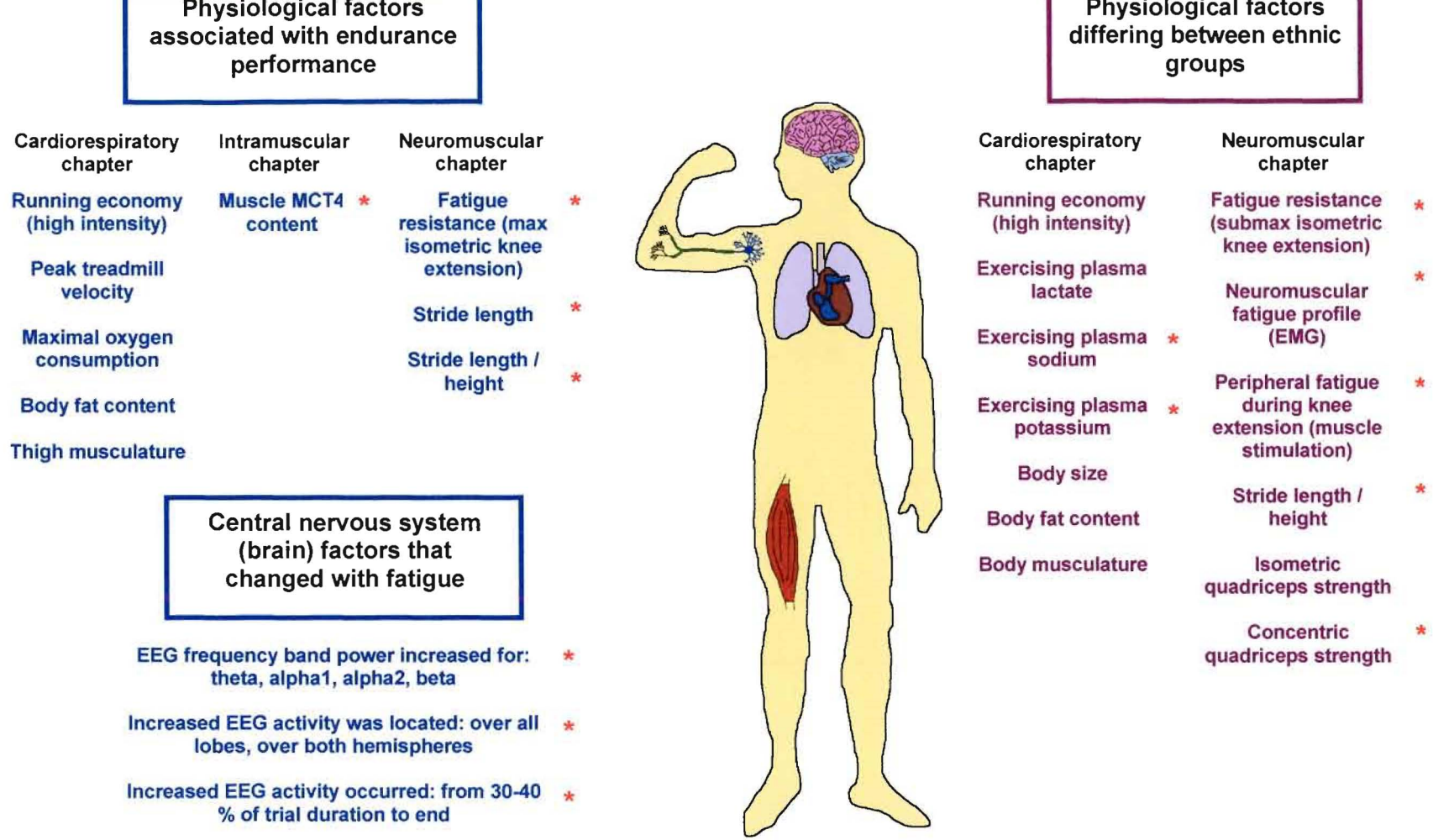


Figure 6.1: Summary of the cardiorespiratory, intramuscular, neuromuscular and central nervous factors that were found to be associated with endurance performance or fatigue, or that were different between black and white South African runners. MCT4: monocarboxylate transporter 4. * indicates novel findings

6.4 METHODS

As described in the introduction of this chapter, multiple regression analyses were performed integrating variables from the Cardiorespiratory, Intramuscular and Neuromuscular factors chapters (Chapters 2, 3 and 4), and using 10 km PB as the outcome variable to be modeled. The variables from Chapters 2, 3 and 4 that were used were chosen based on specific groupings, and were all significantly individually related to 10 km PB. The groups of variables examined included (1) physiological or performance data obtained at rest, (2) physiological data obtained during maximal running testing, (3) physiological or performance data obtained during submaximal running, and (4) the physiological variable from each chapter that best correlated with PB. The multiple regression equation format described in the introduction of this chapter was used for the modeled data in the results. In addition to the equation, the R^2 value has been given, to indicate how well the model fits the data, or how well the model predicts the dependent variable.

The multiple regression technique used was forward stepwise regression analysis. The independent variables included in the analyses were only incorporated into the final equations if they added significantly to the predictive value of the model at the $p<0.05$ level. Forward stepwise regression may over-estimate the importance of each variable and the goodness of fit if large numbers of variables and small sample sizes are used. It has been recommended that no more than n/10 variables be included in an analysis, where n is sample size [6]. Thirty-two subjects were tested in Chapters 2 to 4, however the effective number of observations was often smaller due to missing data (as detailed in the Statistical analysis sections of the respective chapters). Only two or three independent variables were therefore included in each regression analysis.

While the R^2 value generated with an equation gives an indication of the goodness of fit of the model for the data overall, it does not necessarily indicate how well the model predicts the outcome variable for individuals. Examination of the residuals (the average difference between the observed and predicted y values) from the equation allows one to determine if the model fits the data equally well throughout the range of dependent variable values. Normal probability plots of the residuals were therefore performed for the regression analyses in order to demonstrate this.

6.5 RESULTS

There were a number of physiological or performance variables that correlated significantly with 10 km personal best time (PB) for the cardiorespiratory, intramuscular and neuromuscular systems (Chapters 2, 3 and 4, Table 6.1). This relationship could not be extended to the central nervous system (Chapter 5) as different subjects (who were not all runners) were used in this chapter.

Table 6.1: Physiological and performance variables that correlated significantly with 10 km personal best time.

Variable	Measurement	r value	p value	Chapter
Maximal running performance	PTV	-0.6802	0.001	2
Anthropometry	% Body fat	0.6445	0.001	2
	SSS	0.6516	0.001	
	LTV	-0.3998	0.05	
	LTV/LBM	-0.5752	0.01	
	Endomorphy	0.6010	0.01	
Maximal oxygen consumption	VO_2max	-0.6142	0.01	2
Running economy	$VO_2(kg^{0.66})@12$	0.4972	0.01	2
	$VO_2(kg^{0.66})@14$	0.4852	0.05	
	$VO_2(kg^{0.66})@16$	0.4260	0.05	
Monocarboxylate transporter content	MCT4	-0.5655	0.05	3
Fatiguing static exercise performance	TTFmax	-0.4230	0.05	4
Stride biomechanics	SL	-0.4069	0.05	4
	SL/H	-0.4928	0.01	

PTV: peak treadmill velocity; SSS: sum of seven skinfolds; LTV: lean thigh volume; LBM: lean body mass; TTFmax: time to fatigue during maximal isometric contraction; SL: stride length; SL: stride length/height

The following multiple regression models (Equations 6.1 to 6.4) all include 10 km PB as the outcome (dependent) variable.

6.5.1 Resting data

For the multiple regression analysis of physiological variables measured at rest that were significantly individually correlated with 10 km PB, there was a choice of six

variables (Table 6.1), of which only three could be included in the regression analysis based on sample size (as described in the methods section of this chapter). These included five anthropometrical variables and monocarboxylate transporter 4 content (MCT4). Three of the anthropometrical variables were related to body fat content (% body fat, sum of seven skinfolds (SSS) and endomorphy), and two were related to body musculature (lean thigh volume (LTV) and the ratio of lean thigh volume to lean body mass (LTV/LMB)). As the three body fat content variables are related to each other and the two body muscle variables are related to each other they are likely to account for similar variance in running performance, and therefore only one variable from each of these two groups was included in the regression analysis. Of the three body fat content variables, SSS had the strongest correlation with PB, and of the two body muscle variables, LTV/LBM had the strongest correlation with PB. The multiple regression analysis of physiological data measured at rest therefore included the following predictor variables: SSS, LTV/LBM and MCT4. The analysis excluded LTV/LBM (not significant) and yielded the following equation:

Equation 6.1: Regression model of resting physiological data with 10 km personal best time (n=19).

$$PB = 1940 + 6.37 \times SSS - 0.02 \times MCT4$$

$$R^2 = 0.61 \text{ (adjusted } R^2 = 0.56)$$

$$p<0.001$$

6.5.2 Maximal running test data

For the multiple regression analysis of physiological or performance data obtained during maximal running testing that were significantly individually correlated with 10 km PB, there was a choice of only two variables, namely peak treadmill velocity (PTV) and maximal oxygen consumption (VO_2max, Table 6.1). These two variables were therefore included in the regression analysis as predictor variables. The analysis excluded VO_2max and yielded the following equation:

Equation 6.2: Regression model of maximal running test data with 10 km personal best time (n=23).

$$PB = 4112 - 96.1 \times PTV$$

$$R^2 = 0.47 \text{ (adjusted } R^2 = 0.44)$$

$$p<0.001$$

6.5.3 Submaximal running test data

For the multiple regression analysis of physiological or performance data obtained during submaximal running testing that were significantly individually correlated with 10 km PB, there was a choice of five variables (Table 6.1). These included running economy measured at three different speeds, stride length (SL) and stride length per height (SL/H). Of the two stride length-related variables, SL/H was selected for inclusion in the regression analysis because it had a stronger correlation with PB than SL did. Of the three running economy measurements, running economy (with VO_2 expressed per $kg^{0.66}$ of body mass) measured at 12 km/hr ($VO_2(kg^{0.66})@12$) was selected, partly because it had the strongest correlation with PB and partly because stride length was also measured at 12 km/hr. The regression analysis therefore included SL/H and $VO_2$12k0.66 as predictor variables, and yielded the following equation:

Equation 6.3: Regression model of submaximal physiological data with 10 km personal best time (n=27).

$$PB = 2460 + 4.94 \times VO_2(kg^{0.66})@12 - 913 \times SL/H$$

$$R^2 = 0.39 \text{ (adjusted } R^2 = 0.34)$$

$$p<0.01$$

6.5.4 'Best-correlated' physiological data

The multiple regression analysis of the physiological variable (not performance variables such as PTV) from each of the Cardiorespiratory, Intramuscular and Neuromuscular factors chapters (Chapters 2, 3 and 4) that best correlated with 10 km PB included the following predictor variables: SSS, MCT4 and SL/H. The analysis excluded SL/H and yielded the following equation:

Equation 6.4: Regression model of best-correlated physiological data from Chapters 2, 3 and 4 with 10 km personal best time (n=19).

$$PB = 1940 + 6.37 \times SSS - 0.02 \times MCT4$$

$$R^2 = 0.61 \text{ (adjusted } R^2 = 0.56)$$

$$P<0.001$$

6.5.5 Normal probability plot of residuals

A normal probability plot for the residual values (difference between observed and predicted values) from Equation 6.1 (resting data) is shown in Figure 6.2. The normal probability plots for Equations 6.2, 6.3 and 6.4 each consisted of a similar 'straight' curve (data not shown).

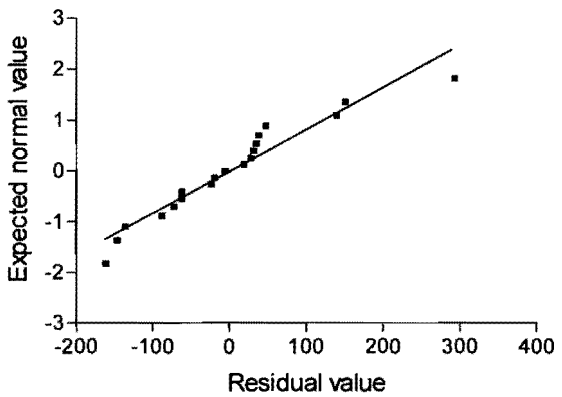


Figure 6.2. Normal probability plot of residuals for Equation 6.1 (resting data).

6.6 DISCUSSION

As suggested by many authors, no single factor can alone account for fatigue or for variations in endurance performance [23,65,122,157,255]. This is evident in Table 6.1, which shows that multiple variables measured in this thesis have been shown to correlate significantly with endurance performance. In addition, the premise that fatigue involves many different mechanisms from different physiological systems [65,122,141,255] is consistent with the finding that there are variables from the cardiorespiratory (Chapter 2), intramuscular (Chapter 3) and neuromuscular (Chapter 4) systems that are significantly related to endurance performance (Table 6.1). Although the importance of integrative research in exercise physiology is acknowledged by many researchers, few integrative studies are actually conducted, probably because this type of research is not as straightforward and does not necessarily progress as rapidly as more reductionist approaches [182]. In addition, research integrating multiple physiological factors often requires a greater sample size, resulting in greater time and cost. Due to the limited amount of integrative research that is being conducted, work in this area can add significantly to the understanding of fatigue and endurance performance.

The multiple regression analyses also suggest that many physiological variables are involved in endurance performance, and indicate that factors measured during different types of physiological testing can be relevant to endurance activity. The first regression equation (6.1) shows the importance of physiological data that is measured from the resting body without exercise testing. While the lean thigh volume to lean body mass ratio was excluded from the equation, the sum of seven skinfolds and the monocarboxylate transporter 4 content were included. The R^2 value of 0.61 indicates that this model predicts 61 % of the variance in 10 km running performance. Therefore, the body fat content and the total muscle content of monocarboxylate transporter 4 can predict 61 % of the variance in 10 km running performance in well-trained runners. That said, it is likely that this value is an overestimation. Forward stepwise regression can over-estimate the importance of each variable and the goodness of fit of the model if large numbers of variables and small sample sizes are used. Although only three variables were included in the analysis and only two in the final equation, the sample size for this analysis was only 19, as a result of only 19 monocarboxylate transporter

assays being performed. However, this findings suggests that resting factors in the body can be involved in performance during non-resting, exercise conditions.

In order to prevent overestimation of the importance of the variables in a multiple regression model, it is advisable to include large subject numbers in the sample, especially when a large number of variables are going to be examined. However, multiple regression analysis including many variables is often used in studies with a small sample size [6]. For example, in a study of the factors affecting perceived exertion during prolonged exercise in hot environments, Nybo and Nielsen [345] included 5 independent variables in a forward stepwise regression analysis with 14 subjects. Although this can render the regression model unstable, if the limitations of the model are discussed and the results interpreted with prudence, the analysis can be used with a small sample size.

The second equation (6.2) resulted from an analysis of data obtained during maximal running testing. VO_2max and PTV were included in the analysis, and VO_2max was excluded from the final model. As a result the equation contains only one predictor variable, PTV, and essentially takes the form of a simple linear regression model. The multiple correlation coefficient (R^2) indicated a predictive capacity of 47 %, suggesting that PTV can account for 47 % of the variance in 10 km running performance in trained distance runners. VO_2max may have been excluded from the final model because it accounted for similar variance in PB as PTV did. As many variables measured during exercise testing are related and influence each other, some of them are likely to account for similar or the same variance in the prediction equation. As such, one of them may in effect cancel another out of the multiple regression equation. Physiological factors involved in the same functional systems in the body are often involved in determining related attributes of physical ability and are therefore perhaps more likely to account for similar variance in the regression equation. This again emphasises the importance of measuring variables from a variety of physiological systems when attempting to predict fatigue resistance or athletic performance.

The third equation (6.3) resulted from an analysis of data obtained during submaximal, rather than maximal, running testing. Stride length per height and running economy measured at 12 km/hr were included in this model, and resulted in an R^2 value of 0.39,

suggesting that these two variables can together account for 39 % of the variance in 10 km running performance. This is a greater predictive value than that obtained by either of these two variables if correlated with PB separately (r^2 = 0.24 and 0.25 for SL//H and $VO_2(kg^{0.66})$@12, respectively). Therefore, while maximal running testing is regularly used as an indicator of endurance ability, submaximal testing also has value. This is to be expected, seeing as endurance exercise is performed at a submaximal level.

The fourth equation (6.4) included the physiological variable from each of the Cardiorespiratory, Intramuscular and Neuromuscular factors chapters that best correlated with 10 km PB. The sum of seven skinfolds, muscle MCT4 content and the stride length to height ratio were therefore used in the analysis. The analysis excluded SL/H and, interestingly, yielded the same model as equation one for the resting physiological variables (6.1). The R^2 value indicates that these two predictor variables can explain 61 % of the variance in 10 km running performance. While measuring muscle MCT proteins is an invasive test requiring specific laboratory equipment, the skinfolds measurement is a simple, noninvasive process requiring only skinfold calipers. It is interesting to observe that simple anthropometrical measurements are perhaps just as important in exercise performance studies as the ones made possible by more complicated technology.

It should be noted that the sum of seven skinfolds is not actually a cardiorespiratory variable, despite being included in the Cardiorespiratory factors chapter. However, in the context of this chapter, this is not important, as the key issue is that many physiological systems are involved in fatigue, rather than the particular roles of specific variables. Together, these regression equations demonstrate that factors measured at rest, during submaximal exercise and during maximal exercise are all associated with endurance performance.

Clement et al [83] similarly used multiple regression analysis to identify factors associated with distance running ability, specifically incorporating performance criteria as the independent or predictor variables in the models. They found that an equation including vertical jump height and distance run in 12 minutes could distinguish good runners from elite runners. They also found that, within an elite group of runners, distance running ability could be predicted by running efficiency at submaximal speeds. The finding that

vertical jump height is related to distance running performance is not in agreement with the data from Chapter 4 of this thesis, which indicated that vertical jump height was not significantly related to running performance. The running efficiency finding of Clement et al [83], however, is in agreement with the result from Chapter 2 of this thesis that running economy at submaximal speeds is significantly correlated with running performance (Table 6.1). As shown by the third equation in this chapter, however, the predictive value of running economy for distance running performance can be improved with the addition of another physiological variable, in this case the stride length to height ratio. This emphasises that no single factor is of sole importance for endurance performance.

In addition, a series of significant multiple regression equations was formed in the results section of this chapter with different physiological variables, indicating that endurance performance or fatigue resistance is complex, and the importance of a single significant regression equation must be viewed with caution. The regulation of homeostasis and fatigue in the body may be viewed as a 'complex system', with different physiological factors and different fatigue processes exerting interactive effects on each other [255]. The implication of this is that the more variables that are involved in the system, the more random an output variable, such as endurance performance, will appear. The different physiological variables involved in the system may affect each other in ways that may or may not yet be understood, and the generation of a model to perfectly estimate endurance performance is extremely complicated.

As a large number of physiological factors are likely to be involved in fatigue resistance or endurance performance, for a multiple regression equation to accurately estimate performance, it would need to contain a large number of independent variables. However, in order for this to be statistically viable, a very large sample size is necessary. Collecting measurements of many different physiological variables from large subject numbers can prove difficult in terms of time and funding. It is therefore suggested that the pool of potential predictor variables first be narrowed using simple linear regression analysis (as demonstrated in Chapters 2, 3 and 4) with manageable subject numbers. Once a smaller select pool of independent variables, which demonstrate a significant correlation with the dependent variable individually, has been identified, then these variables can be applied to a multiple regression analysis with a much larger sample size. These covariates can then be used to form a regression equation that estimates,

with a certain degree of accuracy, an index of fatigue resistance or endurance performance. This technique was demonstrated in the methods and results sections of this chapter, however a much greater sample size would be necessary to include the huge number of physiological variables involved in the fatigue process.

Both the simple regression and multiple regression techniques used in this thesis have been employed using the assumption that the outcome variable is linearly dependent on the predictor variables. However, there may well be non-linear processes occurring during exercise and fatigue [412]. This is also dependent on the frequency with which measurements are recorded. For example, a variable may appear to be increasing in a linear manner when infrequent measurements are recorded, while a greater sampling frequency reveals the occurrence of small oscillations. Both the large-scale linear increase and the small-scale oscillations could be relevant to the fatigue process. Non-linear statistics may therefore also be useful when relating physiological variables to endurance performance and fatigue resistance. As the nature of the relationship between the dependent and independent variables is not necessarily known a priori, one can use regression procedures that do not make any assumption as to how the dependent variable is related to the predictors, and instead allow the relationship to be determined from the data.

One of the limitations of all regression techniques, however, is that, while relationships can be identified, the underlying causal mechanisms cannot. It is therefore necessary to be prudent when concluding whether a change in a physiological variable actually contributes to the physical symptoms of fatigue or the sensation of fatigue, or if it is merely a concurrent change with the progression of fatigue that is not necessarily directly associated with it.

Normal probability plots of the residuals from the equations were performed to determine if the models fit the data equally well throughout the range of dependent variable values. For the normal probability plot for the first equation (Figure 6.2) the curve is 'straight', indicating that the residuals have an approximately normal distribution and that the model fits the data throughout the range [6]. This implies that this model could be applied to any individual included within the range of subjects tested in the generation of the equation, in other words, well trained distance runners. The normal probability plots for

the other three equations each consisted of a similar 'straight' curve, again indicating that the residuals had a normal distribution, with the models fitting the data throughout the range (data not shown).

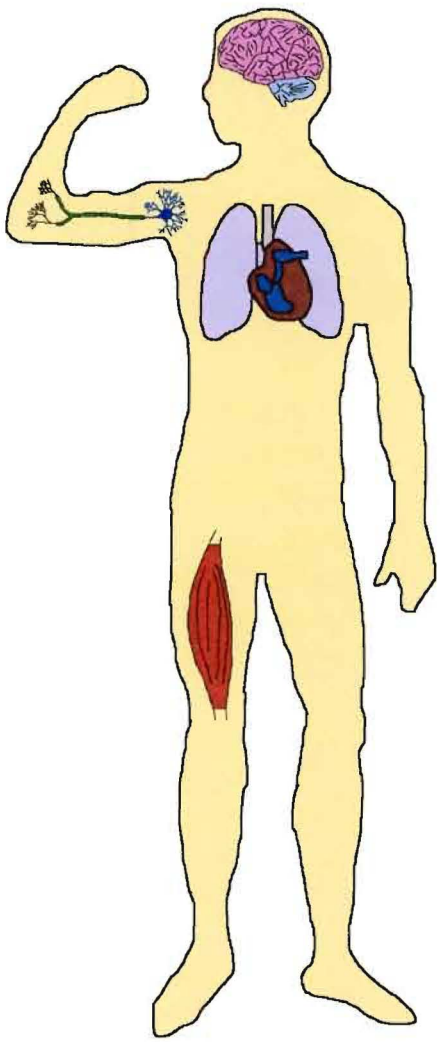
In order for a relative homeostasis to be maintained in the body during fatiguing exercise, the information from the many physiological variables involved in the fatigue process needs to be linked so that there is a coordinated fatigue response. Activity of the central nervous system is likely to be integral to this. Unfortunately, while the CNS fatigue data presented in Chapter 5 of this thesis is relevant to the integrative method of this chapter, the variables from Chapter 5 could not be included in the regression analyses used, as a different set of subjects was tested in Chapter 5, many of whom were not runners. Afferent physiological information from receptors in the different tissues in the body is probably integrated in the CNS to allow the detection of homeostatic imbalances, the generation of the sensation of fatigue and the coordination of the response to fatigue [255,414]. The sensation of fatigue and the centrally coordinated motor response to it may play a crucial role in protecting the body from potential damage were exercise to continue at a certain intensity [340,414].

6.7 CONCLUSIONS

The findings of this chapter are summarised in Figure 6.3. Many physiological variables measured in this thesis, from the cardiorespiratory, intramuscular and neuromuscular systems, correlated significantly with endurance performance. This is consistent with the premise that endurance activity, and hence fatigue, involves multiple mechanisms from different physiological systems. Multiple regression analysis also suggests that many factors are involved in endurance performance, including variables measured from the body at rest, and those that can be recorded during maximal or submaximal exercise testing. The interaction of different physiological variables with each other should be taken into account when examining each of their individual relationships with fatigue. The results of this chapter indicate that endurance performance or fatigue resistance is complex, and is associated with the integrative functioning of multiple factors from different physiological systems.

Physiological systems and endurance performance

Multiple regression and endurance performance



Which physiological systems are involved in endurance performance?

Cardiorespiratory system

Intramuscular system

Neuromuscular system

(Central nervous system - although not analysed in this chapter)

Which types of measured data are associated with endurance performance?

Resting data

Submaximal exercise data

Maximal exercise data

Figure 6.3: The integration of physiological variables and systems in endurance performance.

Chapter 7 Summary and conclusions

7.1 FOREWORD

The objective of this thesis was to assess the hypothesis that any single physiological factor or system can wholly account for fatigue during physical activity, by examining whether fatigue instead involves numerous factors from different physiological systems in the body. This thesis therefore investigated the relationships between fatigue and multiple parts of the human physiology during endurance performance. In addition, it was hoped that novel associations between endurance performance and physiological variables could be uncovered, and that potential reasons for the superior performance of black over white South African runners could be elaborated on. As detailed in the introduction, endurance performance has been used as a proxy for fatigue resistance, seeing as these two factors can be regarded as analogous, in practical terms, and due to fatigue itself not necessarily consisting of anything measurable. Hence, the title of this thesis: "Endurance performance: the integrative physiology of resisting fatigue".

The findings of this thesis have revealed several novel results and confirmed previously reported results regarding the physiology of endurance performance, and therefore fatigue resistance. Cardiorespiratory, intramuscular and neuromuscular factors all correlated with endurance performance, and there was activity in multiple brain regions common to subjects during fatiguing exercise. This is inconsistent with the theory that any single system can be the sole source of fatigue, and consistent with the premise that fatigue involves multiple mechanisms from different physiological systems. This research also found that multiple physiological or performance variables were significantly different between black and white South African runners, suggesting that the observed endurance performance difference between these ethnic groups may result from many, rather than a single, physiological factor. These major findings, and the conclusions relating to these findings, will now be discussed. Lastly, a model of the progression of the physiological changes associated with fatigue during physical activity is proposed, and suggestions for future investigations are offered.

7.2 NOVEL FINDINGS

The most important finding of this thesis is that multiple different physiological and performance variables, associated with different physiological systems, are involved in endurance performance, and hence fatigue resistance. This finding results from an amalgamation of the individual results from the different chapters of the thesis. Again combining the findings from the different chapters, it is evident that within the different physiological systems, multiple factors are involved in fatigue during endurance activity. For example, there was activity in multiple different regions of the brain during fatiguing exercise. There were also multiple physiological or performance variables that were significantly different between black and white South African runners matched for 10 km performance ability, again suggesting that the processes and mechanisms involved in fatigue during endurance performance are complex. In addition, the factors that were different between the two ethnic groups, and the factors that were significantly related to 10 km running performance, were varied in nature, suggesting that integration of considerably differing factors is occurring during fatigue-associated processes during endurance activity.

Three very different variables were found to be significantly associated with 10 km running performance for the athletes tested in this thesis. One was a physiological variable in the muscle, one was a performance measure and one was a biomechanical measure. The intramuscular factor was skeletal muscle MCT4 content. The novel finding is that the greater the MCT4 content in the vastus lateralis muscle of the runners, the better the race performance. This is suggested to be due to enhanced MCT4-mediated efflux of lactate from the muscle cell, allowing the continuation of glycolysis during exercise, as well as the associated efficient efflux of H^+ ions, which would delay fatigue by preventing high levels of acidity in the cell. The performance measure that correlated significantly with 10 km running performance was the time to task failure during a maximal quadriceps isometric fatigue test. This suggests that the ability to resist fatigue and therefore perform well during static exercise is associated with the ability to perform well during dynamic exercise, at least in trained distance runners. The biomechanical measures of stride length and stride length per height were also positively associated with 10 km running ability, possibly due to a function of the power, or the elasticity or stiffness, in the leg muscles. While the beneficial effect of a greater stride length has

been reported before for distances of 1500 m and 3000 m [54], it has not, to the knowledge of this author, been shown for 10 km performance.

The discovery that brain activity changed significantly during fatiguing exercise in multiple frequency bands, over many areas of the brain and in both cortical hemispheres, in a manner common to 19 individuals, is a novel finding. It is considered probable that fatigue is linked to multiple brain regions and processes, and it is proposed that a 'fatigue matrix' exists in the brain, encompassing the network of brain areas that is activated with fatigue. As many of these regions in the brain are likely to be common to people during fatiguing physical activity, their detection is possible, and it is suggested that future studies attempt to identify these regions using techniques such as electroencephalography, regional cerebral blood flow and nuclear magnetic resonance imaging.

There were also several novel findings with respect to the physiological and performance differences or similarities between black and white South African distance runners. These are summarised in Table 7.1, along with findings confirming results from previous studies. For clarification, the ethnic origins of these two groups will once again be defined: the "black South African" runners studied in this thesis were all males living in the Western Cape of South Africa, of Southern African descent, with the majority being descended from the Xhosa tribe. The "White South African" runners were all Caucasian males living in the Western Cape of South Africa, of European descent (including English, Scottish, Irish, German and Dutch).

Table 7.1: Summary of differences and similarities in physiological and performance variables found between black and white South African runners who were matched for 10 km race performance.

Physiological/performance variable	Comparison	
Height and weight	black < white	
Body muscle content	black > white	
Body fat content	black ≤ white	
Lean thigh volume/Lean body mass	black > white	
Maximal oxygen consumption	black = white	
Peak treadmill velocity	black = white	
Running economy	black ≥ white	
Heart rate	black = white	
Respiratory exchange ratio	black = white	
Plasma lactate	black < white	
Plasma sodium	black ≤ white	†
Plasma potassium	black ≥ white	†
Muscle fibre composition	black = white	
Muscle monocarboxylate transporter content	black = white	†
Isometric quadriceps strength	black < white	
Concentric quadriceps strength	black < white	†
Eccentric quadriceps strength	black = white	†
Sustained isometric fatigue resistance	black > white	†
Neuromuscular activity (EMG) changes with fatigue	black < white	†
Peripheral fatigue during isometric exercise (stimulation)	black < white	†
Stretch-shortening cycle efficiency	black = white	†
Stride length/Height	black > white	†

black < white indicate that the value obtained for the black subjects was lower than that for the white subjects; black > white indicate that the value obtained for the black subjects was higher than that for the white subjects; ≤ and ≥ indicate that more than one similar measurement was made (e.g. at different exercise intensities) and only one/some were significantly different between groups. † indicates novel findings.

Black distance runners are known to outperform their white counterparts in competition [47,84,338], and therefore any physiological differences between them may be related to this observed performance difference, as well as potentially to the difference in endurance ability between people in general. Investigation of the cardiorespiratory differences between black and white South African runners revealed that many of the physiological differences between these ethnic groups were only apparent at higher exercise

intensities, which would be relevant during 10 km competition when the athletes are performing at these high intensities. In addition, and which has not been shown before, there were differences between the two groups in their plasma sodium and potassium levels during maximal exercise. This suggests that the sodium/potassium flux may be different in black and white South African runners, although whether this would translate to differences within the muscle as well still needs to be determined.

It has previously, and again in this thesis, been found that black South African runners have lower exercising plasma lactate levels than the white runners. It has been shown in this thesis for the first time, however, that this is unlikely to be due a difference between the groups in the total cellular density of MCT1 or MCT4 in the muscle (or the relationship between their densities). This is based on the finding that neither MCT1 content, MCT4 content nor the ratio of the two was significantly different between the black and the white runners. As only the total muscle content of these lactate transporters was measured, however, an ethnic comparison of the expression of these MCT's in the mitochondrial and sarcolemmal fractions is recommended for future study.

A further novel finding is that the black runners performed significantly better than the white runners during a sustained submaximal isometric quadriceps fatigue test. This suggests that the superior fatigue resistance of black South African runners is not restricted to running or even dynamic exercise, but occurs during static exercise as well. Electromyographic changes accompanying isometric fatigue, which have not previously been compared in these ethnic groups, suggested less peripheral fatigue in the black compared to the white runners, as did force output responses to muscle stimulation. The efficiency of stretch-shortening cycle functioning, also not previously compared, was not different between the two ethnic groups, and it is therefore probable that it is not a difference in elastic energy utilisation that allows black South African runners to perform better than white. On the other hand, another novel finding, that the black runners covered more distance per stride for their height when running than the white runners, suggests that a difference in biomechanics or muscle power could be related to the superior performance of black compared to white South African runners.

The finding that multiple physiological or performance variables were significantly different between black and white South African runners with similar 10 km performance

ability suggests that the endurance performance difference often observed between these ethnic groups may result from many, rather than a single, physiological factor. Similarly, the fact that variables as different as stride biomechanics and muscle protein content both correlate with running ability, and that a large number of brain areas are active with the progression of fatigue, imply that fatigue is a complex phenomenon, involving many different factors or mechanisms.

7.3 ADDITIONAL MAIN FINDINGS

While not novel findings, this thesis confirmed previous reports that peak treadmill velocity and VO_2max are associated with 10 km running performance, with peak treadmill velocity correlating slightly better than VO_2max. Running economy also correlated with 10 km running time, when VO_2 was expressed per $kg^{0.66}$ of body mass. This implies that having a large maximal oxygen consumption and being able to exercise in a metabolically economical manner, as well as being able to achieve a high maximal workload, are associated with endurance ability. In addition, anthropometric variables related to body fat content were also significantly associated with running performance.

Using a greater sample size, this thesis also confirmed previously reported differences between black and white South African runners (summarised in Table 7.1). As found previously, the black athletes had many anthropometrical differences compared to the white athletes, including body mass and height, body fat content, body muscle content and a different relative lean thigh volume. While maximal oxygen consumption was not different between the groups, the black runners were more economical than the white at higher running intensities, when VO_2 was expressed per $kg^{0.66}$ of body mass. As mentioned earlier, in agreement with previous research, exercising plasma lactate levels were lower in the black athletes than the white athletes. Previous reports of similar muscle fibre compositions between the ethnic groups were also confirmed.

The novel relationships found between physiological variables and endurance performance, as well as the previously reported findings that the results of this thesis confirm, together show that many physiological factors, from the cardiorespiratory, intramuscular and neuromuscular systems are associated with endurance performance. This is consistent with the premise that endurance activity, and hence fatigue, involves the integrated functioning of multiple mechanisms from different physiological systems. Multiple regression analysis also suggests that many factors are involved in endurance performance, including variables measured from the body at rest, and those that can be recorded during maximal or submaximal exercise testing. The findings of this thesis therefore indicate that endurance performance or fatigue resistance is complex, and it is speculated that the reason for the involvement of multiple physiological systems in

fatigue during endurance activity is the effective maintenance of relative homeostasis in the body.

7.4 QUESTIONS AND ANSWERS

In an attempt to demonstrate whether fatigue during endurance performance does indeed involve the integrated functioning of multiple physiological factors and systems, the objectives of this thesis included a set of questions relevant to physiological activity during endurance performance. These questions will now be restated and answered, based on the thesis findings.

- Are multiple physiological factors associated with endurance performance?

Yes. Multiple different physiological factors were significantly related to endurance performance, and therefore fatigue resistance. In addition there were significant changes in brain activity during fatiguing exercise that were common to many subjects, suggesting an association of specific brain activity with endurance performance.

- Are multiple physiological systems involved in endurance performance?

Yes. Factors from all of the three physiological systems that correlation analyses were conducted for in this thesis were significantly related to endurance performance.

- Are the cardiorespiratory, intramuscular, neuromuscular and central nervous systems involved in endurance performance?

Yes. Cardiorespiratory, intramuscular and neuromuscular factors were all significantly associated with endurance performance. In addition, common brain activity was found between many individuals during fatiguing exercise, and therefore the central nervous system must also be involved in fatigue resistance, and therefore endurance performance.

- Which of the measured factors from the cardiorespiratory, intramuscular and neuromuscular systems are associated with endurance performance?

Maximal oxygen consumption, running economy, muscle monocarboxylate transporter 4 content, stride length and the stride length to height ratio, as well as the anthropometrical variables percentage body fat, sum of seven skinfolds, lean thigh volume, the lean thigh volume to lean body mass ratio and endomorphy were all significantly associated with endurance performance.

- Which of the measured factors from the cardiorespiratory, intramuscular and neuromuscular systems are different between black and white South African distance runners?

Exercising levels of plasma lactate, sodium and potassium, running economy, the stride length to height ratio, quadriceps strength, neuromuscular recruitment with fatigue and multiple anthropometrical variables (see Table 6.2 for details) were significantly different between black and white South African distance runners.

And therefore:

- Is a single physiological factor likely to wholly account for fatigue during physical activity?

No. The findings of this thesis are inconsistent with the theory that any single physiological factor can be the sole source of fatigue during physical activity.

- Are the factors from a single physiological system likely to wholly account for fatigue during physical activity?

No. The findings of this thesis are inconsistent with the theory that the factors from only one physiological system can form the sole source of fatigue during physical activity.

And for speculation:

- What is the physiological reason for fatigue during physical activity?

It is possible that fatigue may serve a protective function in the body. The sensation of fatigue during physical activity indicates that there is an imbalance in the body's homeostatic state, and allows the fatiguing individual to respond in a manner that may help to correct the imbalance (by reducing the exercise intensity), before they potentially harm their body.

- Why would multiple factors and systems be involved in fatigue during physical activity?

It is possible that the reason for the involvement of multiple factors and systems in fatigue during physical activity is the maintenance of physiological homeostasis, and so that no single metabolic system progresses to failure during the activity. Having a large number of different variables involved in feedforward and feedback control could increase the gain of this homeostatic system, making it more robust.

- How can the findings of this thesis be applied to athletes training to improve their fatigue resistance?

Two interlinked, but different factors are involved in performing well in endurance activity: (1) being proficient at resisting physiological changes to your baseline homeostatic state during exercise (essentially being physically 'fit') – this would involve mainly peripheral factors; and (2) being proficient at causing the physiological changes during exercise to deviate to a large extent from your homeostatic baseline (essentially having the 'will' to push yourself to your maximum) – this would involve mainly central factors. In other words, a 'weak' athlete will physiologically deviate from resting homeostasis easily during exercise, but will not be able to deviate to a large extent or for very long. A 'strong' athlete will not deviate easily from homeostasis, but will be able to deviate to a large extent or maintain the deviation for a long time. Therefore, if two athletes initially have identical ability, athlete A has two ways of potentially improving himself so that he would be able to outperform athlete B. The first would be to physically train himself to become fitter than athlete B. By doing this he would be altering the fatigue responses of the numerous metabolic factors involved in endurance performance so that he could resist physiological changes to homeostasis better, i.e. the same absolute perturbation applied to each would seem relatively less for athlete A. The second method would be for athlete A to mentally train himself to be able to endure the neural fatigue sensations, presumably generated in his cortex and that result from physiological changes to homeostasis, better than athlete B. In this manner, even if they still had the same level of physical fitness, athlete A could withstand a greater absolute perturbation than athlete B. The degree to which an athlete should extend himself beyond the point where his fatigue sensations are extreme is debatable. While overriding the protective sensations may allow him to perform better, it may also result in bodily harm. In practice, an athlete in training will probably be applying both of these 'physical' and 'mental' or 'peripheral' and 'central' methods, even if he is only consciously concentrating on the physical training.

7.5 PROPOSED MODEL OF FATIGUE

A proposed model of the progression of the physiological changes associated with fatigue during exercise has been created based on speculation on the findings of this thesis and previously published literature (Figure 7.1). The model shows the physiological progression of fatigue, from the decision to initiate exercise through to the decision to terminate exercise. While many physiological systems are involved in most of the steps shown in the model, the beginning and ending steps specifically indicate the role of the brain. It should also be noted that, while the model has been designed to show the progression of fatigue and, as such, is of a unidirectional nature, multiple feedforward and feedback loops between the many factors and systems would be occurring throughout the process, and these have not been included in the proposed model.

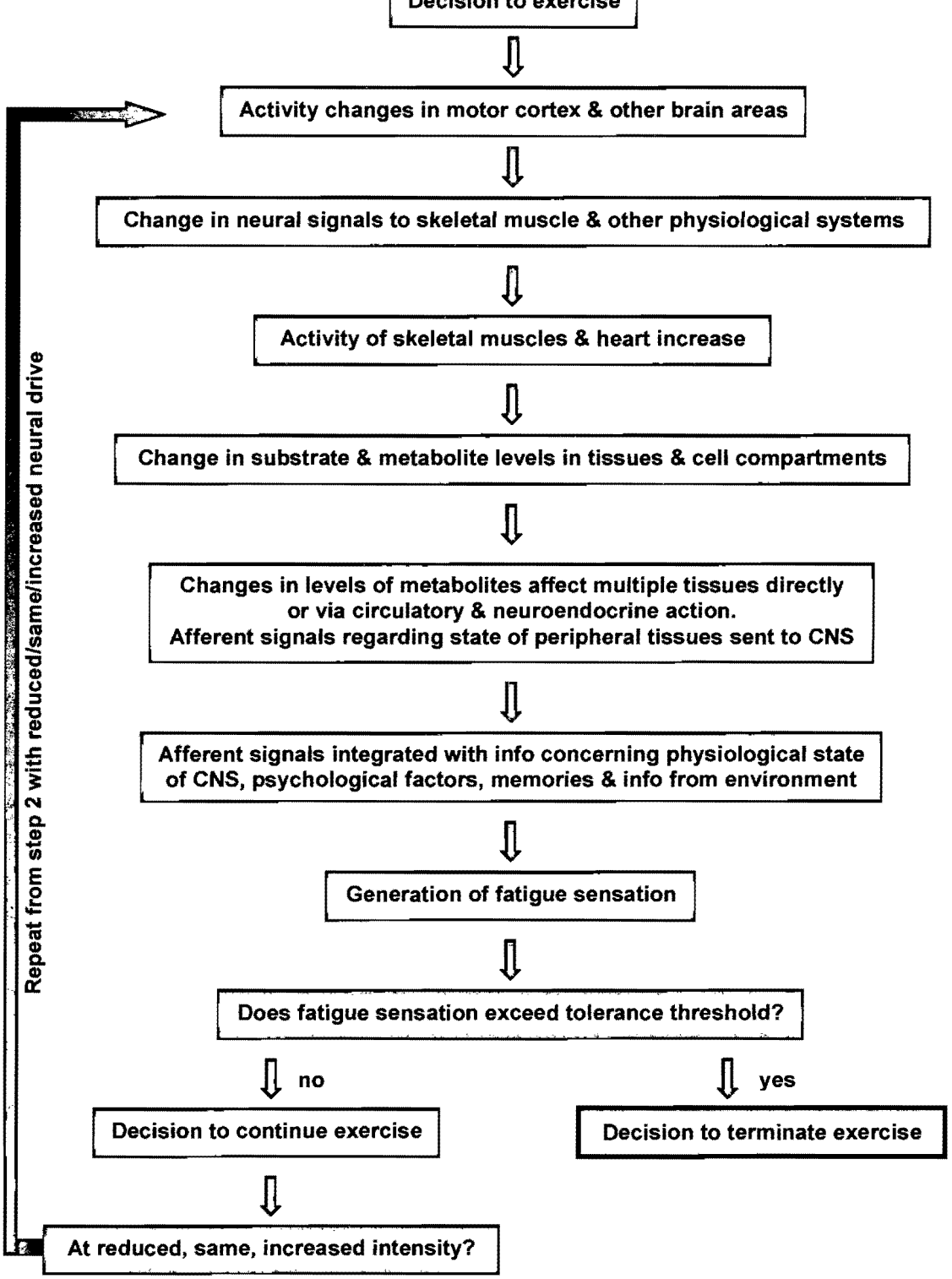


Figure 7.1: Proposed model of fatigue during physical activity

At this point it is appropriate to readdress the issue that the definition of fatigue is still controversial. Fatigue can be viewed as a form of physical failure, as a symptom reported by the exercising individual, or as an entity that consists of the various

physiological processes occurring during endurance activity. Fatigue can also be regarded as a sensation that reflects the conscious awareness of physiological changes, or even as a sensation that functions as an active regulator of exercise intensity, instead of simply being a passive consequence of physiological processes. As such, fatigue can play both a negative and a positive role during exercise. Fatigue has a negative effect on short-term physical performance and a positive effect on long-term performance. Fatigue can prevent an individual from performing well during an endurance activity, which is negative, however fatigue can also prevent the individual from harming their body to the extent that they can never perform the activity again, which is positive. In this proposed model, fatigue is viewed as a sensation that reflects the various physiological processes occurring during endurance activity, and that actively regulates the physical activity, thereby serving as a link between the subconscious and the conscious homeostatic control mechanisms in the human body during exercise.

7.6 FUTURE RESEARCH

The findings of this thesis have identified many avenues for future research, both in terms of elucidating the physiological processes involved in endurance performance and fatigue, and in terms of discovering reasons for the superior performance of black South African distance runners. The recurrent finding of low exercising blood lactate levels in black African athletes is still not completely explained, and it is suggested that an ethnic comparison of the expression of skeletal muscle MCT's in the subcellular mitochondrial and sarcolemmal fractions be conducted. In addition, investigation of potential ethnic differences in other proteins and pathways involved in lactate metabolism, such as the muscle concentration and activity of lactate dehydrogenase, is also recommended. The differences found between the ethnic groups in the exercising levels of plasma sodium and potassium warrant further investigation and it is recommended that the sarcolemmal concentrations of the Na^+/K^+ pump be studied in these groups. The finding that the black runners had a greater stride length relative to their height should also be investigated further, considering that this variable was also found to be significantly associated with endurance performance. If this greater stride length is related to a more efficient use of muscle power or biomechanics, then it could potentially explain the better running economy noted in the black runners. In this respect, it may be useful to record stride parameters in association with force and EMG measurements during running. While force and EMG during SSC activity were measured in this thesis using jumping techniques, a more task-specific investigation would involve their measurement during running. In addition, based on the findings of Bosch et al [47] and Marino et al [284,285], further examination of the distribution and regulation of body temperature with exercise is suggested for comparison in black and white African runners.

An important area for future research is the understanding of the alterations in brain activity with fatigue, and how certain regions of the brain are related to the generation of the sensation of fatigue. In this respect it is suggested that source analyses be performed on the data from future EEG work, possibly in combination with nuclear magnetic resonance and positron emission tomography techniques. It could also prove fruitful to conduct corticocortico and corticomuscular coherence analysis with EEG and EMG data, to identify relationships between areas of activity in these tissues. Considering the relevance of fatigue as a sensation to the work on brain activity, it is

suggested that future research include rating of perceived exertion measures in their protocol. This measurement was excluded from the central nervous system investigation in this thesis due to concern that speaking or pointing could influence the EEG signal, however, if this problem can be overcome, the scientific value added by the measure should be considerable.

There are many other areas that are relevant to fatigue research that were beyond the scope of this thesis. There are numerous metabolites not measured in this work that are likely to have an association with the development of fatigue during physical activity, and all of these are worthy of further study. In addition, factors from the endocrine system, biomechanical factors and nutritional factors can all affect endurance performance [147,338] and are still under investigation. In addition, the application of molecular biology techniques to endurance research is still in the early stages, and the relative importance of specific genetic profiles or the regulation of expression of specific genes will probably aid in the identification of beneficial physiological factors for fatigue resistance.

The importance of research that delves deeper into the mechanisms of each of these areas will no doubt prove useful to the understanding of fatigue and endurance performance. In addition to this, however, research that undertakes to combine the findings from all of these separate areas, and describe their integrated functioning during fatigue with physical activity is vital. This research is likely to involve the measurement of large numbers of variables, the testing of large numbers of subjects and the use of both linear and non-linear statistics. Despite practical or technological difficulties, however, the investigation of the intricate and integrative fatigue-related mechanisms during exercise in humans is necessary for a more complete understanding of the complex nature of fatigue.

Appendices

A: INFORMED CONSENT FORMS

Form 1. English informed consent used for the Cardiorespiratory, Intramuscular and Neuromuscular factors chapters (Chapters 2, 3 and 4)

INFORMED CONSENT

Study : The physiological characteristics of high performance South African distance runners.

This study is an attempt to determine physiological factors potentially related to athletic performance in high performance South African distance runners.

I, the undersigned, have been fully informed about the risks inherent in participation in this trial. I also understand that the following measurements/tests may be conducted on myself during this study:

- Maximal treadmill test
- Submaximal treadmill tests
- Muscle strength tests
- Jump tests
- Electromyographic tests
- Body measurement tests
- Blood acquisition
- Muscle biopsy

I understand that blood acquisition and muscle biopsy are invasive and have certain risks. Blood will be drawn from the actecubital vein in the arm. The muscle biopsy involves taking a small piece (100mg – about half the size of a 1 cent coin) of muscle from the vastus lateralis (the outer side of the thigh muscle) via insertion of a biopsy needle into the anaesthetised muscle. A local anaesthetic will be administered prior to the biopsy. The wound will be cleaned and closed with plastic stitches. A small degree of pain will be experienced after the biopsy. Occasional complications include infection (wound sepsis), muscle bruising (haematoma formation) and numbness (peripheral subcutaneous nerve injury). The physician performing the biopsy will carefully explain the procedure and answer any questions before performing the biopsy.

I understand that I will receive an estimate of my VO_{2max}, my quadriceps muscle strength, my percentage body fat and my fibre type upon completion of the study if I should desire it.

I understand that I will be free to withdraw from the study at any time and that I will not be subjected to any pressure whatsoever to remain in the trial. All the information collected during the study will be treated with the strictest confidentiality and will only be used for scientific purposes. I understand that my blood sample will only be used for the purposes explained to me, namely blood lactate, sodium and potassium measurement. Names and personal particulars will not be released under any circumstances. I will be free to ask any questions about the procedures and results of the study.

I, the undersigned, have read and understood the purposes and procedures involved in this scientific study.

Date: _____

Name of subject: _____ Signature: _____

Name of investigator: _____ Signature: _____

Name of witness: _____ Signature: _____

Form 2. Xhosa informed consent used for the Cardiorespiratory, Intramuscular and Neuromuscular factors chapters (Chapters 2, 3 and 4)

IMVUME YOKWENZA UVAVANYO

Ufundo: Ukuqonda ubume bomzimba kwembaleki zomgama omde za se Mzantsi Afrika malunga ne genetiki 'nefisioloji.

Oluvavanyo lwenzele ukuqonda ukuba ingaba kukho umahloko ngokwakheka, kwimbaleki zogqatso olude zase Mzantsi Afrika

Mna, mlesi ndiyavuma ukuba ndixelelwe ngazo zonke ingozi ezinokwenzeka koluvavanyo. Ndiyazi futhi ukuba kuza kuvavanywa oku kulandelayo

- Ukubaleka kwi'treadmill ngesantya sam sonke.
- Ukubaleka kwi'treadmill ngesiqingatha sesantya sam.
- Uvavanyo lwamandla zihlunu
- Uvavanyo lokuxhumela phezulu kangangoko unakho.
- Uvavanyo kukusebenza kwezihlunu zakho ngombane (Electromyographic test).

•Ukumetelwa kwamalungu omzimba.
•Ukutsalwa kwegazi
•Ukuthatyathwa kwentwana yesihlunu sakho ngotyando

Ndiyaqonda ukuba utsalo lwegazi notyando lwesihlunu, kunganengozi njengokuba kungenwa emzimbeni. Igazi lizakutsalwa engalweni. Isihlunu sizakuthathwa ethangeni ngokufakwa kwenaliti kwimasile edonyiweyo kuthathwa isihlunu isiyi 100mg. Ukudonywa kwethanga kuzakwenziwa phambi ko tyando. Isilonda sizakuhlanjwa emvakoko sithungwe. Ubuhlungu obuncinane kuzakuvakala emva kotyando Isihlunu sise nekufuma ubuhlungu, ukugruzuka nenkantsi.
Ugqirha owenza utyando, uyakuyichaza yonke imiqathango yotyando yaye eyakwamkela nemibuzo osabanayo.

Ndiyaqonda ukuba ndikufumana ubuchule malunga nobume bomzimba wam xa ndigqibe uvavanyo ukuba ndiyafuna.

Ndiyaqonda ukuba ndingaphuma nanini na kolu vavanyo yaye andinyanzelekanga ukuba ndiqhubekeke ndakunga phatheki kakuhle.
Iziphumo zoluvavanyo aziyikupapashwa nakubanina koko zakusetyenziswa kwizifundo zobunzululwazi. Ndiyaqonda ukuba igazi endiphise ngalo, liyakusetyenziswa njengoko ndichazelwe umzekelo i"blood lactate", ityiwa yomzimba "potassium" negenetiki (genes).
Amagama kunje nemfihlelo zakho aziyokupapashwa nakubani na. Ndivumelekile ndibuze ngayo yonke inkqubo yoluvavanyo kunye neziphumo zalo.

Mna, ndisayinileyo, ndiyifundile yaye ndayiqonda imfuneko kunye nenkqubo yoluphando.

Umhla.....
Igama lam..... Uphawu.....
Igama lomhleli..... Uphawu.....
Igama lonozakuzaku..... Uphawu.....

Form 3. Informed consent used for the Central nervous factors chapter (Chapter5)

INFORMED CONSENT

Study: An integrative investigation of fatigue using EEG.

This study is an attempt to determine the effect of fatigue on the body's physiology and neural patterns.

I, the undersigned, have been fully informed about the risks inherent in participation in this trial. I also understand that the following measurements/tests may be conducted on myself during this study:

- Muscle strength tests
- Electromyographic tests
- Electroencephalographic tests
- Height and weight measurements
- Oxygen consumption measurements
- Heart rate measurements

I understand that I will receive an estimate of my quadriceps muscle strength, my height and my weight upon completion of the study if I should desire it.

I understand that I will be free to withdraw from the study at any time and that I will not be subjected to any pressure whatsoever to remain in the trial. All the information collected during the study will be treated with the strictest confidentiality and will only be used for scientific purposes. Names and personal particulars will not be released under any circumstances. I will be free to ask any questions about the procedures and results of the study.

I, the undersigned, have read and understood the purposes and procedures involved in this scientific study.

Date: _____

Name of subject: _____ Signature: _____

Name of investigator: _____ Signature: _____

Name of witness: _____ Signature: _____

B: SUBJECT QUESTIONNAIRE

AFRICAN RUNNING STUDY QUESTIONNAIRE

Name: _____

Subject code: _____

Please return to: Yolande Harley
Sports Science Institute of South Africa
Boundary Rd, Newlands

Please don't hesitate to contact me if you have any questions:
650 4559 (o/h)
447 0995 (a/h)

UCT/MRC RESEARCH UNIT FOR EXERCISE SCIENCE AND SPORTS MEDICINE
SPORTS SCIENCE INSTITUTE OF SOUTH AFRICA

Personal details

First name			
Surname			
Phone (day time)			
Age		Date of birth	
Place of birth		ID number	
Nationality		Occupation	
Father's occupation		Mother's occupation	
Ancestry: Tribal or national background (eg Xhosa, Sotho, Zulu, etc)	Father		Unknown ☐
	Mother		Unknown ☐
Smoker?	Yes (current) ☐	Yes (ex-smoker) ☐	No, never ☐
	If yes: Number of years _____		Number per day _____
	If stopped: When _____		
Do you suffer from any medical conditions? eg. Asthma, Diabetes, Heart Disease	Yes ☐		No ☐
	If yes, please specify Heart Disease ☐ Kidney Disease ☐ Lung Disease ☐ Diabetes ☐ Asthma ☐ Other ☐ If other, please specify: _____		
Are you on any medication (over the counter or prescription)?	Yes ☐		No ☐
	If yes, please specify:		
Do you have or have you recently had (last 6 months) any injuries?	Yes ☐		No ☐
	If yes, please describe injury and when it occurred:		
On average how many hours of sleep do you get a night?	Week day: _____ hours Weekend day: _____ hours		

Race history

Please fill in your best race times for each year (where applicable) on the following table:

Year	Best 10km		Best 21.1km			Best 42.2km			Best comrades		
	min	sec	hrs	min	sec	hrs	min	sec	hrs	min	sec
2001											
2000											
1999											
1998											
1997											
1996											
1995											
1994											
1993											
1992											
1991											

C: HISTOLOGICAL MYOSIN ADENOSINE TRIPHOSPHATASE STAINING

Solutions

Solution A, pH 9.4 (Veronal Buffer)

0.1 M Sodium Barbitone	12.5 ml
(MW 206.04; 10.3 g/500 ml)	
0.18 M Calcium Chloride	12.5 ml
(fused granular MW 110.99; 9.99 g/500 ml)	
Distilled water	37.5 ml

Solution B

0.2 N Acetic acid
(1.2 ml / 100 ml distilled water)

Solution C

0.2 M Sodium Acetate
(Anhydrous salt MW 82.03; 16.4 g / 1000 ml)

0.2 M Acetate buffer, pH 4.3

20 ml Solution B
10 ml Solution C

0.2 M Acetate buffer, pH 4.6

10 ml Solution B
20 ml Solution C

ATP solution, pH 9.4 (substrate incubating solution)

0.1 M sodium barbitone	5 ml
0.18 M calcium chloride	2.5 ml
Distilled water	17.5 ml
ATP (disodium salt)	0.075 g

(Place solution in oven at 37 degrees centigrade while cutting sections)

D: SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS OF MYOSIN HEAVY CHAIN ISOFORMS

Solutions

Solution A

20 mM NaCl

3.4 mM NaH_2PO_4

1.6 mM Na_2HPO_4

1 mM EGTA, pH 7.4

Extraction Buffer

100 mM PPiNa, pH 8.5

5 mM EGTA, pH 7.4

1 mM DTT

Gel composition

Separating gel –

- 30 % Glycerol
- 4 % Acrylamide (50:1)
- 0.2 M Tris, pH 8.8
- 0.1 M Glycine
- 0.4 % SDS
- 0.05 % TEMED
- 0.1 % Ammonium Persulphate

Stacking gel –

- 30 % Glycerol
- 4 % Acrylamide (50:1)
- 70 mM Tris, pH 6.8
- 4 mM EDTA, pH 7.0
- 0.4 % SDS
- 0.05 % TEMED
- 0.1 % Ammonium Persulphate

Loading buffer

5 % SDS

20 % Glycerol

125 mM Tris

1 mM EDTA

5 % Beta Mercaptoethanol

0.25 % Bromophenol Blue

Running buffers

Upper buffer –

0.1 M Tris (Base)

150 mM Glycine

0.1 % SDS

Lower buffer –

50 mM Tris (Base)

75 mM Glycine

0.05 % SDS

Tank buffer –

0.5 M Tris (Base)

750 mM Glycine

0.5 % SDS

E: PROTEIN EXTRACTION FOR MCT ANALYSIS

Solutions

Buffer A, pH 7.4

210 mM sucrose
2 mM EGTA
40 mM NaCl
30 mM HEPES

Buffer B, pH 7.4

1.167 M KCl
58.3 mM $Na_4P_2O_7.10\ H_20$

Buffer C, pH 7.4

1 mM EDTA
10 mM Tris

F: WESTERN BLOTTING FOR MCT ANALYSIS

Solutions

Gel composition

Resolving gel –

10 % Acrylamide
0.375 M Tris-Cl, pH 8.8
0.1 % SDS
0.1 % Ammonium Persulphate
0.1 % TEMED

Stacking gel –

4 % Acrylamide
0.175 M Tris-Cl, pH 6.8
0.1 % SDS
0.008 % Phenol Red
0.1 % Ammonium Persulphate
0.1 % TEMED

6X Sample buffer (with DTT)

1 M Tris-Cl, pH 6.8
13.3 % SDS
67 % Glycerol
10.3 % DTT
0.07 % Bromophenol Blue

1X Tank buffer

0.05 M Tris
0.38 M Glycine
0.1 % SDS

Transfer buffer

0.025 M Tris-base

0.19 M Glycine

20 % Methanol

TBS (10X), pH 7.5

500 mM Tris

1.5 M NaCl

TTBS

0.1 % Tween 20

50 mM Tris

150 mM NaCl

Blocking buffer

10 % non-fat dried milk

0.1 % Tween 20

50 mM Tris

150 mM NaCl

Stripping buffer

100 mM ß-mercaptoethanol

60 mM Tris, pH 6.8

2% SDS

Acknowledgements

- My supervisors, Associate Professor Alan St Clair Gibson and Doctor Malcolm Collins of The University of Cape Town and the Medical Research Council of South Africa, respectively, for their enthusiasm and long hours of support.
- Professor Tim Noakes of The University of Cape Town, for his ideas and inspiration, and for providing a wonderful workplace.
- Professor George Brooks of The University of California at Berkeley, whose support, genuine hospitality and scientific intellect I was the fortunate recipient of, as well as for the kind donation of antibodies.
- Doctor Karen Sharwood of The University of Cape Town, for her invaluable and good-natured assistance with subject recruitment and testing.
- Doctor Savita Khanna formerly of The University of California at Berkeley, who taught me laboratory techniques, and about how people work differently in different labs, as well as being a good friend.
- Doctor Liesl Grobler of The University of Cape Town, for kindly donating her time and technical skills in fibre typing.
- Doctor Hugh Mullany formerly of University College Dublin, for his patient explanations of electromyography and his generous provision of EMG and force analysis software.
- Doctor Lance Myers, Associate Professor Vivienne Russell, Associate Professor Mike Lambert, Associate Professor Vicki Lambert and Professor Kit Vaughan for their assistance with various parts of the thesis.
- Sacha West, Jonathan Dugas, Lara Keytel, Amanda Claassen, Regan Arendse and Dudley Tabakin, for their kind assistance with subject testing, as well as Wayne Viljoen, Dale Hudson, Delphine Oliver, Mark Kirkman, Ross Tucker and the other MSc/PhD students of the UCT/MRC Research Unit for Exercise Science and Sports Medicine, for their helpful discussions and camaraderie.
- The students of the Brooks lab at The University of California at Berkeley in 2000, who made my stay a pleasure and reminded me that in the United States 'Cheerio' is a breakfast cereal and not an expression of farewell.

- Lisa Sivewright and Lorraine Sivewright for their assistance with the research administration.
- Musa and Nceba, for kindly giving of their time and skills as Xhosa interpreters.
- The Wellcome Trust, the Harry Crossley and Nellie Atkinson Research Funds of the University of Cape Town, the Medical Research Council of South Africa, Discovery Health and Science Foundation Ireland for research support.
- The Education Abroad Program of the University of Cape Town and The University of California at Berkeley the sponsoring my opportunity to learn new techniques in the United States of America.
- The UCT/MRC Research Unit for Exercise Science and Sports Medicine, the JW Duncan Baxter scholarship, the University Scholarships Committee award and the KW Johnston Bequest Scholarship of the University of Cape Town for financial support.
- Bromor foods and Peninsula beverages for the sponsorship of drinks for subjects.
- The Ethics and Research Committee of the University of Cape Town for ethical clearance.
- Michael Raine, for assistance with diagrams and software, and ALL his other support.
- My family and friends, for always reminding me that one should take one's work seriously, but not oneself.

References

1. (1990) NHLBI Workshop summary. Respiratory muscle fatigue. Report of the Respiratory Muscle Fatigue Workshop Group. *Am. Rev. Respir. Dis.* 142, 474-480.

2. Alegre M., Labarga A., Gurtubay I.G., Iriarte J., Malanda A., and Artieda J. (2003) Movement-related changes in cortical oscillatory activity in ballistic, sustained and negative movements. *Exp. Brain Res.* 148, 17-25.

3. Allen G.M., Gandevia S.C., and McKenzie D.K. (1995) Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. *Muscle Nerve* 18, 593-600.

4. Allen P.J. and Brooks G.A. (1994) Partial purification and reconstitution of the sarcolemmal L-lactate carrier from rat skeletal muscle. *Biochem. J.* 303 (Pt 1), 207-212.

5. Allum J.H., Dietz V., and Freund H.J. (1978) Neuronal mechanisms underlying physiological tremor. *J. Neurophysiol.* 41, 557-571.

6. Altman D.G. (1991) Multiple regression. In Practical statistics for medical research, pp. 336-351. Chapman and Hall, London.

7. Ama P.F., Lagasse P., Bouchard C., and Simoneau J.A. (1990) Anaerobic performances in black and white subjects. *Med. Sci. Sports Exerc.* 22, 508-511.

8. Ama P.F., Simoneau J.A., Boulay M.R., Serresse O., Theriault G., and Bouchard C. (1986) Skeletal muscle characteristics in sedentary black and Caucasian males. *J. Appl. Physiol* 61, 1758-1761.

9. Aura O. and Komi P.V. (1986) Effects of prestretch intensity on mechanical efficiency of positive work and on elastic behavior of skeletal muscle in stretch-shortening cycle exercise. *Int. J. Sports Med.* 7, 137-143.

10. Avela J. and Komi P.V. (1998) Interaction between muscle stiffness and stretch reflex sensitivity after long-term stretch-shortening cycle exercise. *Muscle Nerve* 21, 1224-1227.

11. Baddeley A. (1996) The fractionation of working memory. *Proc. Natl. Acad. Sci. U. S. A* 93, 13468-13472.

12. Baddeley A. (1998) Working memory. *C. R. Acad. Sci. III* 321, 167-173.

13. Baddeley A. (2000) The episodic buffer: a new component of working memory? *Trends Cogn Sci.* 4, 417-423.

14. Baker S.J. and Hardy L. (1989) Effects of high intensity canoeing training on fibre area and fibre type in the latissimus dorsi muscle. *Br. J. Sports Med.* 23, 23-26.

15. Baker S.K., McCullagh K.J., and Bonen A. (1998) Training intensity-dependent and tissue-specific increases in lactate uptake and MCT-1 in heart and muscle. *J. Appl. Physiol* 84, 987-994.

16. Bale P., Bradbury D., and Colley E. (1986) Anthropometric and training variables related to 10km running performance. *Br. J. Sports Med.* 20, 170-173.

17. Bangsbo J., Juel C., Hellsten Y., and Saltin B. (1997) Dissociation between lactate and proton exchange in muscle during intense exercise in man. *J. Physiol* 504 (Pt 2), 489-499.

18. Banister E.W. (1979) The perception of effort: an inductive approach. *Eur. J. Appl. Physiol Occup. Physiol* 41, 141-150.

19. Barthel T., Schnittker R., Juneja L.R., Geiss K., Liesen H., and Weiss M. EEG-changes in humans during regeneration after heavy physical strain with the influence of L-Theanine an amino acid in green tea. Amino.Acids 21, 59. 2001.

20. Basar E., Schurmann M., and Sakowitz O. (2001) The selectively distributed theta system: functions. *Int. J. Psychophysiol.* 39, 197-212.

21. Bassett D.R., Jr. (2002) Scientific contributions of A. V. Hill: exercise physiology pioneer. *J. Appl. Physiol* 93, 1567-1582.

22. Berg K., Latin R.W., and Coffey C. (1998) Relationship of somatotype and physical characteristics to distance running performance in middle age runners. *J. Sports Med. Phys. Fitness* 38, 253-257.

23. Bergh U., Ekblom B., and Astrand P.O. (2000) Maximal oxygen uptake "classical" versus "contemporary" viewpoints. *Med. Sci. Sports Exerc.* 32, 85-88.

24. Bergstrom J. (1962) Muscle electrolytes in man. *Scand. J. Clin. Lab. Invest. Med.* 14 Suppl. 68, 511-513.

25. Bergstrom J. and Hultman E. (1966) The effect of exercise on muscle glycogen and electrolytes in normals. *Scand. J. Clin. Lab Invest* 18, 16-20.

26. Bigland-Ritchie B. (1981) EMG and fatigue of human voluntary and stimulated contractions. *Ciba Found. Symp.* 82, 130-156.

27. Bigland-Ritchie B. (1984) Muscle fatigue and the influence of changing neural drive. *Clin. Chest Med.* 5, 21-34.

28. Bigland-Ritchie B., Donovan E.F., and Roussos C.S. (1981) Conduction velocity and EMG power spectrum changes in fatigue of sustained maximal efforts. *J. Appl. Physiol* 51, 1300-1305.

29. Bigland-Ritchie B., Johansson R., Lippold O.C., Smith S., and Woods J.J. (1983) Changes in motoneurone firing rates during sustained maximal voluntary contractions. *J. Physiol* 340, 335-346.

30. Bigland-Ritchie B., Johansson R., Lippold O.C., and Woods J.J. (1983) Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *J. Neurophysiol.* 50, 313-324.

31. Bigland-Ritchie B. and Woods J.J. (1984) Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve* 7, 691-699.

32. Billat V.L., Sirvent P., Py G., Koralsztein J.P., and Mercier J. (2003) The concept of maximal lactate steady state: a bridge between biochemistry, physiology and sport science. *Sports Med.* 33, 407-426.

33. Billeter R., Heizmann C.W., Howald H., and Jenny E. (1981) Analysis of myosin light and heavy chain types in single human skeletal muscle fibers. *Eur. J. Biochem.* 116, 389-395.

34. Billeter R., Heizmann C.W., Reist U., Howald H., and Jenny E. (1981) alpha- and beta-tropomyosin in typed single fibers of human skeletal muscle. *FEBS Lett.* 132, 133-136.

35. Bilodeau M., Arsenault A.B., Gravel D., and Bourbonnais D. (1990) The influence of an increase in the level of force on the EMG power spectrum of elbow extensors. *Eur. J. Appl. Physiol Occup. Physiol* 61, 461-466.

36. Bilodeau M., Cincera M., Arsenault A.B., and Gravel D. (1997) Normality and stationarity of EMG signals of elbow flexor muscles during ramp and step isometric contractions. *J. Electromyogr. Kinesiol.* 7, 87-96.

37. Binder M.C., Bawa P., Ruenzel P., and Henneman E. (1983) Does orderly recruitment of motoneurons depend on the existence of different types of motor units? *Neurosci. Lett.* 36, 55-58.

38. Binkofski F., Amunts K., Stephan K.M., Posse S., Schormann T., Freund H.J., Zilles K., and Seitz R.J. (2000) Broca's region subserves imagery of motion: a combined cytoarchitectonic and fMRI study. *Hum. Brain Mapp.* 11, 273-285.

39. Blanpied P., Levins J.A., and Murphy E. (1995) The effects of different stretch velocities on average force of the shortening phase in the stretch-shorten cycle. *J. Orthop. Sports Phys. Ther.* 21, 345-353.

40. Bobbert M.F., Gerritsen K.G., Litjens M.C., and Van Soest A.J. (1996) Why is countermovement jump height greater than squat jump height? *Med. Sci. Sports Exerc.* 28, 1402-1412.

41. Bonen A. (2000) Lactate transporters (MCT proteins) in heart and skeletal muscles. *Med. Sci. Sports Exerc.* 32, 778-789.

42. Bonen A., McCullagh K.J., Putman C.T., Hultman E., Jones N.L., and Heigenhauser G.J. (1998) Short-term training increases human muscle MCT1 and femoral venous lactate in relation to muscle lactate. *Am. J. Physiol* 274, E102-E107.

43. Bonen A., Miskovic D., Tonouchi M., Lemieux K., Wilson M.C., Marette A., and Halestrap A.P. (2000) Abundance and subcellular distribution of MCT1 and MCT4 in heart and fast-twitch skeletal muscles. *Am. J. Physiol Endocrinol. Metab* 278, E1067-E1077.

44. Bonen A., Tonouchi M., Miskovic D., Heddle C., Heikkila J.J., and Halestrap A.P. (2000) Isoform-specific regulation of the lactate transporters MCT1 and MCT4 by contractile activity. *Am. J. Physiol Endocrinol. Metab* 279, E1131-E1138.

45. Booth F.W., Gordon S.E., Carlson C.J., and Hamilton M.T. (2000) Waging war on modern chronic diseases: primary prevention through exercise biology. *J. Appl. Physiol* 88, 774-787.

46. Booth F.W. and Thomason D.B. (1991) Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. *Physiol Rev.* 71, 541-585.

47. Bosch A.N., Goslin B.R., Noakes T.D., and Dennis S.C. (1990) Physiological differences between black and white runners during a treadmill marathon. *Eur. J. Appl. Physiol Occup. Physiol* 61, 68-72.

48. Bosco C., Komi P.V., and Ito A. (1981) Prestretch potentiation of human skeletal muscle during ballistic movement. *Acta Physiol Scand.* 111, 135-140.

49. Bosco C., Tarkka I., and Komi P.V. (1982) Effect of elastic energy and myoelectrical potentiation of triceps surae during stretch-shortening cycle exercise. *Int. J. Sports Med.* 3, 137-140.

50. Bottinelli R., Canepari M., Pellegrino M.A., and Reggiani C. (1996) Force-velocity properties of human skeletal muscle fibres: myosin heavy chain isoform and temperature dependence. *J. Physiol* 495 (Pt 2), 573-586.

51. Bottinelli R., Pellegrino M.A., Canepari M., Rossi R., and Reggiani C. (1999) Specific contributions of various muscle fibre types to human muscle performance: an in vitro study. *J. Electromyogr. Kinesiol.* 9, 87-95.

52. Botvinick M., Nystrom L.E., Fissell K., Carter C.S., and Cohen J.D. (1999) Conflict monitoring versus selection-for-action in anterior cingulate cortex. *Nature* 402, 179-181.

53. Boutcher S.H. and Landers D.M. (1988) The effects of vigorous exercise on anxiety, heart rate, and alpha activity of runners and nonrunners. *Psychophysiology* 25, 696-702.

54. Brandon L.J. and Boileau R.A. (1992) Influence of metabolic, mechanical and physique variables on middle distance running. *J. Sports Med. Phys. Fitness* 32, 1-9.

55. Brisswalter J. and Legros P. (1994) Daily stability in energy cost of running, respiratory parameters and stride rate among well-trained middle distance runners. *Int. J. Sports Med.* 15, 238-241.

56. Brody L.R., Pollock M.T., Roy S.H., De Luca C.J., and Celli B. (1991) pH-induced effects on median frequency and conduction velocity of the myoelectric signal. *J. Appl. Physiol* 71, 1878-1885.

57. Brooke M.H. and Kaiser K.K. (1970) Muscle fiber types: how many and what kind? *Arch. Neurol.* 23, 369-379.

58. Brooks G.A. (1991) Current concepts in lactate exchange. *Med. Sci. Sports Exerc.* 23, 895-906.

59. Brooks G.A. (1998) Mammalian fuel utilization during sustained exercise. *Comp Biochem. Physiol B Biochem. Mol. Biol.* 120, 89-107.

60. Brooks G.A. (1999) Are arterial, muscle and working limb lactate exchange data obtained on men at altitude consistent with the hypothesis of an intracellular lactate shuttle? *Adv. Exp. Med. Biol.* 474, 185-204.

61. Brooks G.A. (2000) Intra- and extra-cellular lactate shuttles. *Med. Sci. Sports Exerc.* 32, 790-799.

62. Brooks G.A. (2002) Lactate shuttles in nature. *Biochem. Soc. Trans.* 30, 258-264.

63. Brooks G.A., Brown M.A., Butz C.E., Sicurello J.P., and Dubouchaud H. (1999) Cardiac and skeletal muscle mitochondria have a monocarboxylate transporter MCT1. *J. Appl. Physiol* 87, 1713-1718.

64. Brooks G.A., Dubouchaud H., Brown M., Sicurello J.P., and Butz C.E. (1999) Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle. *Proc. Natl. Acad. Sci. U. S. A* 96, 1129-1134.

65. Brooks G.A., Fahey T.D., White T.P., and Baldwin K.M. (2000) Exercise Physiology. Human Bioenergetics and Its Applications. Mayfield Publishing Company, Mountain View.

66. Brooks G.A. and Mercier J. (1994) Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *J. Appl. Physiol* 76, 2253-2261.

67. Brown P. (2000) Cortical drives to human muscle: the Piper and related rhythms. *Prog. Neurobiol.* 60, 97-108.

68. Burden A. and Bartlett R. (1999) Normalisation of EMG amplitude: an evaluation and comparison of old and new methods. *Med. Eng Phys.* 21, 247-257.

69. Burden A.M., Trew M., and Baltzopoulos V. (2003) Normalisation of gait EMGs: a re-examination. *J. Electromyogr. Kinesiol.* 13, 519-532.

70. Burke E.R., Cerny F., Costill D., and Fink W. (1977) Characteristics of skeletal muscle in competitive cyclists. *Med. Sci. Sports* 9, 109-112.

71. Butz C.E., McClelland G.B., and Brooks G.A. (2004) MCT1 confirmed in rat striated muscle mitochondria. *J. Appl. Physiol.*

72. Caffier G., Rehfeldt H., Kramer H., and Mucke R. (1992) Fatigue during sustained maximal voluntary contraction of different muscles in humans: dependence on fibre type and body posture. *Eur. J. Appl. Physiol Occup. Physiol* 64, 237-243.

73. Carpenter L., Poole R.C., and Halestrap A.P. (1996) Cloning and sequencing of the monocarboxylate transporter from mouse Ehrlich Lettre tumour cell confirms its identity as MCT1 and demonstrates that glycosylation is not required for MCT1 function. *Biochim. Biophys. Acta* 1279, 157-163.

74. Carson R.G., Riek S., and Shahbazpour N. (2002) Central and peripheral mediation of human force sensation following eccentric or concentric contractions. *J. Physiol* 539, 913-925.

75. Cavanagh P.R. and Kram R. (1989) Stride length in distance running: velocity, body dimensions, and added mass effects. *Med. Sci. Sports Exerc.* 21, 467-479.

76. Cavanagh P.R. and Williams K.R. (1982) The effect of stride length variation on oxygen uptake during distance running. *Med. Sci. Sports Exerc.* 14, 30-35.

77. Chen A.C. and Herrmann C.S. (2001) Perception of pain coincides with the spatial expansion of electroencephalographic dynamics in human subjects. *Neurosci. Lett.* 297, 183-186.

78. Chilcott L.A. and Shapiro C.M. (1996) The socioeconomic impact of insomnia. An overview. *Pharmacoeconomics.* 10 Suppl 1, 1-14.

79. Claassen A., Noakes T.D., Lambert E.V., and Bosch A.N. (2001) Evidence of differential sensitivity to hypoglycaemia in carbohydrate-depleted subjects. *Med. Sci. Sports Exerc.* 33, S154.

80. Clark S.A., Aughey R.J., Gore C.J., Hahn A.G., Townsend N.E., Kinsman T.A., Chow C.M., McKenna M.J., and Hawley J.A. (2004) Effects of live high, train low hypoxic exposure on lactate metabolism in trained humans. *J. Appl. Physiol* 96, 517-525.

81. Clarys J.P. and Alewaeters K. (2003) Science and sports: a brief history of muscle, motion and ad hoc organizations. *J. Sports Sci.* 21, 669-677.

82. Clausen T. and Nielsen O.B. (1994) The Na+,K(+)-pump and muscle contractility. *Acta Physiol Scand.* 152, 365-373.

83. Clement D.B., Asmundson C., Taunton C., Taunton J.E., Ridley D., and Banister E.W. (1979) The sports scientist's role in identification of performance criteria for distance runners. *Can. J. Appl. Sport Sci.* 4, 143-148.

84. Coetzer P., Noakes T.D., Sanders B., Lambert M.I., Bosch A.N., Wiggins T., and Dennis S.C. (1993) Superior fatigue resistance of elite black South African distance runners. *J. Appl. Physiol* 75, 1822-1827.

85. Collins D.F., Burke D., and Gandevia S.C. (2002) Sustained contractions produced by plateau-like behaviour in human motoneurones. *J. Physiol* 538, 289-301.

86. Connett R.J., Gayeski T.E., and Honig C.R. (1984) Lactate accumulation in fully aerobic, working, dog gracilis muscle. *Am. J. Physiol* 246, H120-H128.

87. Convertino V.A., Keil L.C., Bernauer E.M., and Greenleaf J.E. (1981) Plasma volume, osmolality, vasopressin, and renin activity during graded exercise in man. *J. Appl. Physiol* 50, 123-128.

88. Costill D.L. (1967) The relationship between selected physiological variables and distance running performance. *J. Sports Med. Phys. Fitness* 7, 61-66.

89. Costill D.L., Branam G., Eddy D., and Sparks K. (1971) Determinants of Marathon running success. *Int. Z. Angew. Physiol* 29, 249-254.

90. Coyle E.F. (1999) Physiological determinants of endurance exercise performance. *J. Sci. Med. Sport* 2, 181-189.

91. Craig A.D. (2002) How do you feel? Interoception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.* 3, 655-666.

92. Cresswell A.G. and Loscher W.N. (2000) Significance of peripheral afferent input to the alpha-motoneurone pool for enhancement of tremor during an isometric fatiguing contraction. *Eur. J. Appl. Physiol* 82, 129-136.

93. Dalsgaard M.K., Ide K., Cai Y., Quistorff B., and Secher N.H. (2002) The intent to exercise influences the cerebral O(2)/carbohydrate uptake ratio in humans. *J. Physiol* 540, 681-689.

94. Davidson R.J. (1992) Anterior cerebral asymmetry and the nature of emotion. *Brain Cogn* 20, 125-151.

95. Davidson R.J., Ekman P., Saron C.D., Senulis J.A., and Friesen W.V. (1990) Approach-withdrawal and cerebral asymmetry: emotional expression and brain physiology. I. *J. Pers. Soc. Psychol.* 58, 330-341.

96. Davidson R.J., Jackson D.C., and Kalin N.H. (2000) Emotion, plasticity, context, and regulation: perspectives from affective neuroscience. *Psychol. Bull.* 126, 890-909.

97. Davidson R.J., Marshall J.R., Tomarken A.J., and Henriques J.B. (2000) While a phobic waits: regional brain electrical and autonomic activity in social phobics during anticipation of public speaking. *Biol. Psychiatry* 47, 85-95.

98. Debrand E., Heard E., and Avner P. (1998) Cloning and localization of the murine Xpct gene: evidence for complex rearrangements during the evolution of the region around the Xist gene. *Genomics* 48, 296-303.

99. Decherchi P., Darques J.L., and Jammes Y. (1998) Modifications of afferent activities from Tibialis anterior muscle in rat by tendon vibrations, increase of interstitial potassium or lactate concentration and electrically-induced fatigue. *J. Peripher. Nerv. Syst.* 3, 267-276.

100. Delp M.D., Armstrong R.B., Godfrey D.A., Laughlin M.H., Ross C.D., and Wilkerson M.K. (2001) Exercise increases blood flow to locomotor, vestibular, cardiorespiratory and visual regions of the brain in miniature swine. *J. Physiol* 533, 849-859.

101. Dennis S.C., Bosch A.N., and Noakes T.D. Factors influencing the exponential rise in blood lactate concentration during progressive exercise. Med.Sci.Sports Exerc. 24[5 Supplement], S83-S83. 1992.

102. Dennis S.C. and Noakes T.D. (1999) Advantages of a smaller bodymass in humans when distance-running in warm, humid conditions. *Eur. J. Appl. Physiol Occup. Physiol* 79, 280-284.

103. Dettmers C., Lemon R.N., Stephan K.M., Fink G.R., and Frackowiak R.S. (1996) Cerebral activation during the exertion of sustained static force in man. *Neuroreport* 7, 2103-2110.

104. Dettmers C., Ridding M.C., Stephan K.M., Lemon R.N., Rothwell J.C., and Frackowiak R.S. (1996) Comparison of regional cerebral blood flow with transcranial magnetic stimulation at different forces. *J. Appl. Physiol* 81, 596-603.

105. Deuel N.R. and Park J. (1993) Gallop kinematics of Olympic three-day event horses. *Acta Anat. (Basel)* 146, 168-174.

106. Dhoot G.K. and Perry S.V. (1979) Distribution of polymorphic forms of troponin components and tropomyosin in skeletal muscle. *Nature* 278, 714-718.

107. di Prampero P.E. (1985) Metabolic and circulatory limitations to VO2 max at the whole animal level. *J. Exp. Biol.* 115, 319-331.

108. di Prampero P.E. and Ferretti G. (1990) Factors limiting maximal oxygen consumption in humans. *Respir. Physiol* 80, 113-127.

109. Dimitrijevic M.R., McKay W.B., Sarjanovic I., Sherwood A.M., Svirtlih L., and Vrbova G. (1992) Co-activation of ipsi- and contralateral muscle groups during contraction of ankle dorsiflexors. *J. Neurol. Sci.* 109, 49-55.

110. Dimmer K.S., Friedrich B., Lang F., Deitmer J.W., and Broer S. (2000) The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. *Biochem. J.* 350 Pt 1, 219-227.

111. Dinges D.F. (1995) An overview of sleepiness and accidents. *J. Sleep Res.* 4, 4-14.

112. Dubose L., Schelhorn T.B., and Clamann H.P. (1987) Changes in contractile speed of cat motor units during activity. *Muscle Nerve* 10, 744-752.

113. Dubouchaud H., Butterfield G.E., Wolfel E.E., Bergman B.C., and Brooks G.A. (2000) Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am. J. Physiol Endocrinol. Metab* 278, E571-E579.

114. Durandt J.J. The aerobic exercise capacity of sedentary black and white young adults. 1995. Honours thesis.

115. Durnin J.V. and Womersley J. (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br. J. Nutr.* 32, 77-97.

116. Ebenbichler G.R., Kollmitzer J., Erim Z., Loscher W.N., Kerschan K., Posch M., Nowotny T., Kranzl A., Wober C., and Bochdansky T. (2000) Load-dependence of fatigue related changes in tremor around 10 Hz. *Clin. Neurophysiol.* 111, 106-111.

117. Edgerton V.R., Goslow G.E., Jr., Rasmussen S.A., and Spector S.A. (1980) Is resistance of a muscle to fatigue controlled by its motoneurones? *Nature* 285, 589-590.

118. Edwards R.H. (1981) Human muscle function and fatigue. *Ciba Found. Symp.* 82, 1-18.

119. Enerson B.E. and Drewes L.R. (2003) Molecular features, regulation, and function of monocarboxylate transporters: implications for drug delivery. *J. Pharm. Sci.* 92, 1531-1544.

120. Enoka R.M., Robinson G.A., and Kossev A.R. (1989) Task and fatigue effects on low-threshold motor units in human hand muscle. *J. Neurophysiol.* 62, 1344-1359.

121. Enoka R.M. and Stuart D.G. (1985) The contribution of neuroscience to exercise studies. *Fed. Proc.* 44, 2279-2285.

122. Enoka R.M. and Stuart D.G. (1992) Neurobiology of muscle fatigue. *J. Appl. Physiol* 72, 1631-1648.

123. Enoki T., Yoshida Y., Hatta H., and Bonen A. (2003) Exercise training alleviates MCT1 and MCT4 reductions in heart and skeletal muscles of STZ-induced diabetic rats. *J. Appl. Physiol* 94, 2433-2438.

124. Evans M. and Amiel S.A. (1998) Carbohydrates as a cerebral metabolic fuel. *J. Pediatr. Endocrinol. Metab* 11 Suppl 1, 99-102.

125. Evans W.J., Phinney S.D., and Young V.R. (1982) Suction applied to a muscle biopsy maximizes sample size. *Med. Sci. Sports Exerc.* 14, 101-102.

126. Evertsen F., Medbo J.I., and Bonen A. (2001) Effect of training intensity on muscle lactate transporters and lactate threshold of cross-country skiers. *Acta Physiol Scand.* 173, 195-205.

127. Eydoux N., Dubouchaud H., Py G., Granier P., Prefaut C., and Mercier J. (2000) Lactate transport in rat sarcolemmal vesicles after a single bout of submaximal exercise. *Int. J. Sports Med.* 21, 393-399.

128. Eydoux N., Py G., Lambert K., Dubouchaud H., Prefaut C., and Mercier J. (2000) Training does not protect against exhaustive exercise-induced lactate transport capacity alterations. *Am. J. Physiol Endocrinol. Metab* 278, E1045-E1052.

129. Farina D. and Merletti R. (2000) Comparison of algorithms for estimation of EMG variables during voluntary isometric contractions. *J. Electromyogr. Kinesiol.* 10, 337-349.

130. Farina D., Merletti R., and Enoka R.M. (2004) The extraction of neural strategies from the surface EMG. *J. Appl. Physiol* 96, 1486-1495.

131. Farley C.T., Blickhan R., Saito J., and Taylor C.R. (1991) Hopping frequency in humans: a test of how springs set stride frequency in bouncing gaits. *J. Appl. Physiol* 71, 2127-2132.

132. Farley C.T. and Gonzalez O. (1996) Leg stiffness and stride frequency in human running. *J. Biomech.* 29, 181-186.

133. Farley C.T. and Morgenroth D.C. (1999) Leg stiffness primarily depends on ankle stiffness during human hopping. *J. Biomech.* 32, 267-273.

134. Farrell S.W., Kohl H.W., and Rogers T. (1987) The independent effect of ethnicity on cardiovascular fitness. *Hum. Biol.* 59, 657-666.

135. Ferdjallah M., Wertsch J.J., and Shaker R. (2000) Spectral analysis of surface electromyography (EMG) of upper esophageal sphincter-opening muscles during head lift exercise. *J. Rehabil. Res. Dev.* 37, 335-340.

136. Fink W.J., Costill D.L., and Pollock M.L. (1977) Submaximal and maximal working capacity of elite distance runners. Part II. Muscle fiber composition and enzyme activities. *Ann. N. Y. Acad. Sci.* 301, 323-327.

137. Finsterer J. (2001) EMG-interference pattern analysis. *J. Electromyogr. Kinesiol.* 11, 231-246.

138. Fisch B.J. (1999) Fisch and Spehlmann's EEG primer, basic principles of digital and analog EEG, pp. 148-149. Elsevier, Amsterdam.

139. Fishbein W.N. (1986) Lactate transporter defect: a new disease of muscle. *Science* 234, 1254-1256.

140. Fishbein W.N., Merezhinskaya N., and Foellmer J.W. (2002) Relative distribution of three major lactate transporters in frozen human tissues and their localization in unfixed skeletal muscle. *Muscle Nerve* 26, 101-112.

141. Fitts R.H. (1994) Cellular mechanisms of muscle fatigue. *Physiol Rev.* 74, 49-94.

142. Fitts R.H. (1996) Muscle fatigue: the cellular aspects. *Am. J. Sports Med.* 24, S9-13.

143. Florence J.M. and Hagberg J.M. (1984) Effect of training on the exercise responses of neuromuscular disease patients. *Med. Sci. Sports Exerc.* 16, 460-465.

144. Foster C. (2003) ACSM and the emergence of the profession of exercise physiologist. *Med. Sci. Sports Exerc.* 35, 1247.

145. Foster C., Cadwell K., Crenshaw B., Dehart-Beverley M., Hatcher S., Karlsdottir A.E., Shafer N.N., Theusch C., and Porcari J.P. (2001) Physical activity and exercise training prescriptions for patients. *Cardiol. Clin.* 19, 447-457.

146. Foster C., Costill D.L., Daniels J.T., and Fink W.J. (1978) Skeletal muscle enzyme activity, fiber composition and VO2 max in relation to distance running performance. *Eur. J. Appl. Physiol Occup. Physiol* 39, 73-80.

147. Foster C., Costill D.L., and Fink W.J. (1979) Effects of preexercise feedings on endurance performance. *Med. Sci. Sports* 11, 1-5.

148. Freyssenet D., Berthon P., Denis C., Barthelemy J.C., Guezennec C.Y., and Chatard J.C. (1996) Effect of a 6-week endurance training programme and branched-chain amino acid supplementation on histomorphometric characteristics of aged human muscle. *Arch. Physiol Biochem.* 104, 157-162.

149. Friesema E.C., Ganguly S., Abdalla A., Manning Fox J.E., Halestrap A.P., and Visser T.J. (2003) Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J. Biol. Chem.* 278, 40128-40135.

150. Fu K.M., Foxe J.J., Murray M.M., Higgins B.A., Javitt D.C., and Schroeder C.E. (2001) Attention-dependent suppression of distracter visual input can be cross-modally cued as indexed by anticipatory parieto-occipital alpha-band oscillations. *Brain Res. Cogn Brain Res.* 12, 145-152.

151. Fuglevand A.J., Zackowski K.M., Huey K.A., and Enoka R.M. (1993) Impairment of neuromuscular propagation during human fatiguing contractions at submaximal forces. *J. Physiol* 460, 549-572.

152. Fukashiro S., Abe T., Shibayama A., and Brechue W.F. (2002) Comparison of viscoelastic characteristics in triceps surae between Black and White athletes. *Acta Physiol Scand.* 175, 183-187.

153. Fukashiro S. and Komi P.V. (1987) Joint moment and mechanical power flow of the lower limb during vertical jump. *Int. J. Sports Med.* 8 Suppl 1, 15-21.

154. Fukashiro S., Komi P.V., Jarvinen M., and Miyashita M. (1995) In vivo Achilles tendon loading during jumping in humans. *Eur. J. Appl. Physiol Occup. Physiol* 71, 453-458.

155. Gandevia S.C. (1982) The perception of motor commands or effort during muscular paralysis. *Brain* 105, 151-159.

156. Gandevia S.C. (1999) Mind, muscles and motoneurones. *J. Sci. Med. Sport* 2, 167-180.

157. Gandevia S.C. (2001) Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev.* 81, 1725-1789.

158. Gandevia S.C., Allen G.M., Butler J.E., and Taylor J.L. (1996) Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J. Physiol* 490 (Pt 2), 529-536.

159. Ganong W.F. (1995) Review of medical physiology. Appleton & Lange, Conneticut.

160. Garcia C.K., Brown M.S., Pathak R.K., and Goldstein J.L. (1995) cDNA cloning of MCT2, a second monocarboxylate transporter expressed in different cells than MCT1. *J. Biol. Chem.* 270, 1843-1849.

161. Garcia C.K., Goldstein J.L., Pathak R.K., Anderson R.G., and Brown M.S. (1994) Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. *Cell* 76, 865-873.

162. Garcia C.K., Li X., Luna J., and Francke U. (1994) cDNA cloning of the human monocarboxylate transporter 1 and chromosomal localization of the SLC16A1 locus to 1p13.2-p12. *Genomics* 23, 500-503.

163. Garland S.J., Garner S.H., and McComas A.J. (1988) Reduced voluntary electromyographic activity after fatiguing stimulation of human muscle. *J. Physiol* 401, 547-556.

164. Gasser T., Sroka L., and Mocks J. (1985) The transfer of EOG activity into the EEG for eyes open and closed. *Electroencephalogr. Clin. Neurophysiol.* 61, 181-193.

165. Gerdle B., Edstrom M., and Rahm M. (1993) Fatigue in the shoulder muscles during static work at two different torque levels. *Clin. Physiol* 13, 469-482.

166. Gerdle B., Eriksson N.E., and Hagberg C. (1988) Changes in the surface electromyogram during increasing isometric shoulder forward flexions. *Eur. J. Appl. Physiol Occup. Physiol* 57, 404-408.

167. Gerdle B. and Fugl-Meyer A.R. (1992) Is the mean power frequency shift of the EMG a selective indicator of fatigue of the fast twitch motor units? *Acta Physiol Scand.* 145, 129-138.

168. Gerdle B., Karlsson S., Crenshaw A.G., and Friden J. (1997) The relationships between EMG and muscle morphology throughout sustained static knee extension at two submaximal force levels. *Acta Physiol Scand.* 160, 341-351.

169. Gladden L.B. (2000) Muscle as a consumer of lactate. *Med. Sci. Sports Exerc.* 32, 764-771.

170. Gladden L.B. (2001) Lactic acid: New roles in a new millennium. *Proc. Natl. Acad. Sci. U. S. A* 98, 395-397.

171. Gollhofer A., Horstmann G.A., Schmidtbleicher D., and Schonthal D. (1990) Reproducibility of electromyographic patterns in stretch-shortening type contractions. *Eur. J. Appl. Physiol Occup. Physiol* 60, 7-14.

172. Gollhofer A., Komi P.V., Miyashita M., and Aura O. (1987) Fatigue during stretch-shortening cycle exercises: changes in mechanical performance of human skeletal muscle. *Int. J. Sports Med.* 8, 71-78.

173. Gollnick P.D. and Saltin B. (1982) Significance of skeletal muscle oxidative enzyme enhancement with endurance training. *Clin. Physiol* 2, 1-12.

174. Green H., Dahly A., Shoemaker K., Goreham C., Bombardier E., and Ball-Burnett M. (1999) Serial effects of high-resistance and prolonged endurance training on Na+-K+ pump concentration and enzymatic activities in human vastus lateralis. *Acta Physiol Scand.* 165, 177-184.

175. Green H., Halestrap A., Mockett C., O'Toole D., Grant S., and Ouyang J. (2002) Increases in muscle MCT are associated with reductions in muscle lactate after a single exercise session in humans. *Am. J. Physiol Endocrinol. Metab* 282, E154-E160.

176. Green H.J., Chin E.R., Ball-Burnett M., and Ranney D. (1993) Increases in human skeletal muscle Na(+)-K(+)-ATPase concentration with short-term training. *Am. J. Physiol* 264, C1538-C1541.

177. Green H.J. and Patla A.E. (1992) Maximal aerobic power: neuromuscular and metabolic considerations. *Med. Sci. Sports Exerc.* 24, 38-46.

178. Hakkinen K., Komi P.V., and Kauhanen H. (1986) Electromyographic and force production characteristics of leg extensor muscles of elite weight lifters during isometric, concentric, and various stretch-shortening cycle exercises. *Int. J. Sports Med.* 7, 144-151.

179. Halestrap A.P. and Price N.T. (1999) The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. *Biochem. J.* 343 Pt 2, 281-299.

180. Haller R.G., Clausen T., and Vissing J. (1998) Reduced levels of skeletal muscle Na+K+ -ATPase in McArdle disease. *Neurology* 50, 37-40.

181. Hamalainen N. and Pette D. (1995) Patterns of myosin isoforms in mammalian skeletal muscle fibres. *Microsc. Res. Tech.* 30, 381-389.

182. Hamilton M.T. and Booth F.W. (2000) Skeletal muscle adaptation to exercise: a century of progress. *J. Appl. Physiol* 88, 327-331.

183. Hampson D.B., St Clair G.A., Lambert M.I., and Noakes T.D. (2001) The influence of sensory cues on the perception of exertion during exercise and central regulation of exercise performance. *Sports Med.* 31, 935-952.

184. Harmon-Jones E. and Allen J.J. (1998) Anger and frontal brain activity: EEG asymmetry consistent with approach motivation despite negative affective valence. *J. Pers. Soc. Psychol.* 74, 1310-1316.

185. Haug B.A., Baudewig J., and Paulus W. (1998) Selective activation of human cortical area V5A by a rotating visual stimulus in fMRI; implication of attentional mechanisms. *Neuroreport* 9, 611-614.

186. Heath B.H. and Carter J.E. (1967) A modified somatotype method. *Am. J. Phys. Anthropol.* 27, 57-74.

187. Helgeson K. and Gajdosik R.L. (1993) The stretch-shortening cycle of the quadriceps femoris muscle group measured by isokinetic dynamometry. *J. Orthop. Sports Phys. Ther.* 17, 17-23.

188. Hellsing G. and Lindstrom L. (1983) Rotation of synergistic activity during isometric jaw closing muscle contraction in man. *Acta Physiol Scand.* 118, 203-207.

189. Henneman E. (1957) Relation between size of neurons and their susceptibility to discharge. *Science* 126, 1345-1347.

190. Hickie I.B., Hooker A.W., Hadzi-Pavlovic D., Bennett B.K., Wilson A.J., and Lloyd A.R. (1996) Fatigue in selected primary care settings: sociodemographic and psychiatric correlates. *Med. J. Aust.* 164, 585-588.

191. Hill A.V., Long C.N.H., and Lupton H. (1924) Muscular exercise, lactic acid and the supply and utilization of oxygen. Pt. 1-III. *Proceedings of the Royal Society B* 96, 438-475.

192. Hill A.V., Long C.N.H., and Lupton H. (1924) Muscular exercise, lactic acid and the supply and utilization of oxygen. Pt. VII-IX. *Proceedings of the Royal Society B* 97, 155-176.

193. Hochachka P.W., Stanley C., Matheson G.O., McKenzie D.C., Allen P.S., and Parkhouse W.S. (1991) Metabolic and work efficiencies during exercise in Andean natives. *J. Appl. Physiol* 70, 1720-1730.

194. Horita T., Komi P.V., Nicol C., and Kyrolainen H. (1996) Stretch shortening cycle fatigue: interactions among joint stiffness, reflex, and muscle mechanical performance in the drop jump [corrected]. *Eur. J. Appl. Physiol Occup. Physiol* 73, 393-403.

195. Hortobagyi T., Israel R.G., Houmard J.A., O'Brien K.F., Johns R.A., and Wells J.M. (1992) Comparison of four methods to assess body composition in black and white athletes. *Int. J. Sport Nutr.* 2, 60-74.

196. Howald H., Hoppeler H., Claassen H., Mathieu O., and Straub R. (1985) Influences of endurance training on the ultrastructural composition of the different muscle fiber types in humans. *Pflugers Arch.* 403, 369-376.

197. Hunter A.M., St Clair G.A., Lambert M., Dennis S., Mullany H., O'Malley M.J., Vaughan C.L., Kay D., and Noakes T.D. (2003) EMG amplitude in maximal and submaximal exercise is dependent on signal capture rate. *Int. J. Sports Med.* 24, 83-89.

198. Hunter A.M., St Clair G.A., Lambert M., and Noakes T.D. (2002) Electromyographic (EMG) normalization method for cycle fatigue protocols. *Med. Sci. Sports Exerc.* 34, 857-861.

199. Hunter A.M., St Clair G.A., Lambert M.I., Nobbs L., and Noakes T.D. (2003) Effects of supramaximal exercise on the electromyographic signal. *Br. J. Sports Med.* 37, 296-299.

200. Hunter A.M., St Clair G.A., Mbambo Z., Lambert M.I., and Noakes T.D. (2002) The effects of heat stress on neuromuscular activity during endurance exercise. *Pflugers Arch.* 444, 738-743.

201. Ide K., Schmalbruch I.K., Quistorff B., Horn A., and Secher N.H. (2000) Lactate, glucose and O2 uptake in human brain during recovery from maximal exercise. *J. Physiol* 522 Pt 1, 159-164.

202. IKAI M. and STEINHAUS A.H. (1961) Some factors modifying the expression of human strength. *J. Appl. Physiol* 16, 157-163.

203. Immke D.C. and McCleskey E.W. (2001) Lactate enhances the acid-sensing Na+ channel on ischemia-sensing neurons. *Nat. Neurosci.* 4, 869-870.

204. Ingjer F. (1979) Capillary supply and mitochondrial content of different skeletal muscle fiber types in untrained and endurance-trained men. A histochemical and ultrastructural study. *Eur. J. Appl. Physiol Occup. Physiol* 40, 197-209.

205. Izumi H., Torigoe T., Ishiguchi H., Uramoto H., Yoshida Y., Tanabe M., Ise T., Murakami T., Yoshida T., Nomoto M., and Kohno K. (2003) Cellular pH regulators: potentially promising molecular targets for cancer chemotherapy. *Cancer Treat. Rev.* 29, 541-549.

206. Jackson V.N., Price N.T., Carpenter L., and Halestrap A.P. (1997) Cloning of the monocarboxylate transporter isoform MCT2 from rat testis provides evidence that expression in tissues is species-specific and may involve post-transcriptional regulation. *Biochem. J.* 324 (Pt 2), 447-453.

207. Jackson V.N., Price N.T., and Halestrap A.P. (1995) cDNA cloning of MCT1, a monocarboxylate transporter from rat skeletal muscle. *Biochim. Biophys. Acta* 1238, 193-196.

208. Johnston I.A. (1991) Muscle action during locomotion: a comparative perspective. *J. Exp. Biol.* 160, 167-185.

209. Jonides J., Smith E.E., Koeppe R.A., Awh E., Minoshima S., and Mintun M.A. (1993) Spatial working memory in humans as revealed by PET. *Nature* 363, 623-625.

210. Juel C. (1998) Muscle pH regulation: role of training. *Acta Physiol Scand.* 162, 359-366.

211. Juel C., Bangsbo J., Graham T., and Saltin B. (1990) Lactate and potassium fluxes from human skeletal muscle during and after intense, dynamic, knee extensor exercise. *Acta Physiol Scand.* 140, 147-159.

212. Juel C. and Halestrap A.P. (1999) Lactate transport in skeletal muscle - role and regulation of the monocarboxylate transporter. *J. Physiol* 517 (Pt 3), 633-642.

213. Juel C., Hellsten Y., Saltin B., and Bangsbo J. (1999) Potassium fluxes in contracting human skeletal muscle and red blood cells. *Am. J. Physiol* 276, R184-R188.

214. Juel C., Holten M.K., and Dela F. (2004) Effects of strength training on muscle lactate release and MCT1 and MCT4 content in healthy and type 2 diabetic humans. *J. Physiol* 556, 297-304.

215. Juel C., Klarskov C., Nielsen J.J., Krustrup P., Mohr M., and Bangsbo J. (2004) Effect of high-intensity intermittent training on lactate and H+ release from human skeletal muscle. *Am. J. Physiol Endocrinol. Metab* 286, E245-E251.

216. Juel C., Lundby C., Sander M., Calbet J.A., and Hall G. (2003) Human skeletal muscle and erythrocyte proteins involved in acid-base homeostasis: adaptations to chronic hypoxia. *J. Physiol* 548, 639-648.

217. Juel C., Nielsen J.J., and Bangsbo J. (2000) Exercise-induced translocation of Na(+)-K(+) pump subunits to the plasma membrane in human skeletal muscle. *Am. J. Physiol Regul. Integr. Comp Physiol* 278, R1107-R1110.

218. Juel C. and Pilegaard H. (1998) Lactate/H+ transport kinetics in rat skeletal muscle related to fibre type and changes in transport capacity. *Pflugers Arch.* 436, 560-564.

219. Juel C., Pilegaard H., Nielsen J.J., and Bangsbo J. (2000) Interstitial K(+) in human skeletal muscle during and after dynamic graded exercise determined by microdialysis. *Am. J. Physiol Regul. Integr. Comp Physiol* 278, R400-R406.

220. Kamen G. and Caldwell G.E. (1996) Physiology and interpretation of the electromyogram. *J. Clin. Neurophysiol.* 13, 366-384.

221. Kamp A. and Troost J. (1978) EEG signs of cerebrovascular disorder, using physical exercise as a provocative method. *Electroencephalogr. Clin. Neurophysiol.* 45, 295-298.

222. Kandel E., Schwarts J., and Jessell T. (2000) Principles of neural science. McGraw-Hill, New York.

223. Karlsson J., Sjodin B., Jacobs I., and Kaiser P. (1981) Relevance of muscle fibre type to fatigue in short intense and prolonged exercise in man. *Ciba Found. Symp.* 82, 59-74.

224. Karlsson S. and Gerdle B. (2001) Mean frequency and signal amplitude of the surface EMG of the quadriceps muscles increase with increasing torque--a study using the continuous wavelet transform. *J. Electromyogr. Kinesiol.* 11, 131-140.

225. Katch V.L. and Katch F.I. (1974) A simple antrhopometric method for calculating segmental leg limb volume. *Res. Q.* 45, 211-214.

226. Kay D. and Marino F.E. (2000) Fluid ingestion and exercise hyperthermia: implications for performance, thermoregulation, metabolism and the development of fatigue. *J. Sports Sci.* 18, 71-82.

227. Kay D., Marino F.E., Cannon J., St Clair G.A., Lambert M.I., and Noakes T.D. (2001) Evidence for neuromuscular fatigue during high-intensity cycling in warm, humid conditions. *Eur. J. Appl. Physiol* 84, 115-121.

228. Kay D., St Clair G.A., Mitchell M.J., Lambert M.I., and Noakes T.D. (2000) Different neuromuscular recruitment patterns during eccentric, concentric and isometric contractions. *J. Electromyogr. Kinesiol.* 10, 425-431.

229. Kayser B. (2003) Exercise starts and ends in the brain. *Eur. J. Appl. Physiol* 90, 411-419.

230. Kayser B., Narici M., Binzoni T., Grassi B., and Cerretelli P. (1994) Fatigue and exhaustion in chronic hypobaric hypoxia: influence of exercising muscle mass. *J. Appl. Physiol* 76, 634-640.

231. Kellis E. and Baltzopoulos V. (1998) Muscle activation differences between eccentric and concentric isokinetic exercise. *Med. Sci. Sports Exerc.* 30, 1616-1623.

232. Kent-Braun J.A. (1999) Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. *Eur. J. Appl. Physiol Occup. Physiol* 80, 57-63.

233. Kereshi S., Manzano G., and McComas A.J. (1983) Impulse conduction velocities in human biceps brachii muscles. *Exp. Neurol.* 80, 652-662.

234. Kirk P., Wilson M.C., Heddle C., Brown M.H., Barclay A.N., and Halestrap A.P. (2000) CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. *EMBO J.* 19, 3896-3904.

235. Klimesch W. (1995) Memory processes described as brain oscillations in the EEG-alpha and theta bands. *Psycoloquy* 6.

236. Klimesch W., Doppelmayr M., Russegger H., Pachinger T., and Schwaiger J. (1998) Induced alpha band power changes in the human EEG and attention. *Neurosci. Lett.* 244, 73-76.

237. Knapik J.J., Staab J.S., and Harman E.A. (1996) Validity of an anthropometric estimate of thigh muscle cross-sectional area. *Med. Sci. Sports Exerc.* 28, 1523-1530.

238. Koehler-Stec E.M., Simpson I.A., Vannucci S.J., Landschulz K.T., and Landschulz W.H. (1998) Monocarboxylate transporter expression in mouse brain. *Am. J. Physiol* 275, E516-E524.

239. Komi P.V. (1992) Stretch-shortening cycle. In Strength and power in sports (ed. Komi P.V.), pp. 169-179. Blackwell, Oxford.

240. Komi P.V. and Bosco C. (1978) Utilization of stored elastic energy in leg extensor muscles by men and women. *Med. Sci. Sports* 10, 261-265.

241. Komi P.V. and Tesch P. (1979) EMG frequency spectrum, muscle structure, and fatigue during dynamic contractions in man. *Eur. J. Appl. Physiol Occup. Physiol* 42, 41-50.

242. Komi P.V. and Vitasalo J.H. (1976) Signal characteristics of EMG at different levels of muscle tension. *Acta Physiol Scand.* 96, 267-276.

243. Korzeniewski B. and Zoladz J.A. (2002) Influence of rapid changes in cytosolic pH on oxidative phosphorylation in skeletal muscle: theoretical studies. *Biochem. J.* 365, 249-258.

244. Kram R. and Taylor C.R. (1990) Energetics of running: a new perspective. *Nature* 346, 265-267.

245. Krause G., Ullsperger P., Beyer L., and Gille H.G. (1983) Changes in EEG power density spectrum during static muscle work. *Eur. J. Appl. Physiol Occup. Physiol* 51, 61-66.

246. Kristeva-Feige R., Fritsch C., Timmer J., and Lucking C.H. (2002) Effects of attention and precision of exerted force on beta range EEG-EMG synchronization during a maintained motor contraction task. *Clin. Neurophysiol.* 113, 124-131.

247. Kubitz K.A. and Mott A.A. (1996) EEG power spectral densities during and after cycle ergometer exercise. *Res. Q. Exerc. Sport* 67, 91-96.

248. Kubo K., Kanehisa H., Kawakami Y., and Fukunaga T. (2000) Elastic properties of muscle-tendon complex in long-distance runners. *Eur. J. Appl. Physiol* 81, 181-187.

249. Kubo K., Kawakami Y., and Fukunaga T. (1999) Influence of elastic properties of tendon structures on jump performance in humans. *J. Appl. Physiol* 87, 2090-2096.

250. Kyrolainen H., Finni T., Avela J., and Komi P.V. (2003) Neuromuscular behaviour of the triceps surae muscle-tendon complex during running and jumping. *Int. J. Sports Med.* 24, 153-155.

251. Kyrolainen H., Kivela R., Koskinen S., McBride J., Andersen J.L., Takala T., Sipila S., and Komi P.V. (2003) Interrelationships between muscle structure, muscle strength, and running economy. *Med. Sci. Sports Exerc.* 35, 45-49.

252. Kyrolainen H. and Komi P.V. (1994) Neuromuscular performance of lower limbs during voluntary and reflex activity in power- and endurance-trained athletes. *Eur. J. Appl. Physiol Occup. Physiol* 69, 233-239.

253. Kyrolainen H. and Komi P.V. (1995) Differences in mechanical efficiency between power- and endurance-trained athletes while jumping. *Eur. J. Appl. Physiol Occup. Physiol* 70, 36-44.

254. Lafreniere R.G., Carrel L., and Willard H.F. (1994) A novel transmembrane transporter encoded by the XPCT gene in Xq13.2. *Hum. Mol. Genet.* 3, 1133-1139.

255. Lambert E.V., St Clair Gibson A., and Noakes T.D. Complex system model of fatigue: Integral control and defense of homeostasis during exercise in humans. Br.J.Sports Med. 2004. In Press.

256. Lane R.D., Reiman E.M., Bradley M.M., Lang P.J., Ahern G.L., Davidson R.J., and Schwartz G.E. (1997) Neuroanatomical correlates of pleasant and unpleasant emotion. *Neuropsychologia* 35, 1437-1444.

257. Larsson L. and Forsberg A. (1980) Morphological muscle characteristics in rowers. *Can. J. Appl. Sport Sci.* 5, 239-244.

258. Lepers R., Maffiuletti N.A., Rochette L., Brugniaux J., and Millet G.Y. (2002) Neuromuscular fatigue during a long-duration cycling exercise. *J. Appl. Physiol* 92, 1487-1493.

259. Lewis S.F. (1984) Exercise and human neuromuscular diseases: a symposium overview. *Med. Sci. Sports Exerc.* 16, 449-450.

260. Li J.L., Wang X.N., Fraser S.F., Carey M.F., Wrigley T.V., and McKenna M.J. (2002) Effects of fatigue and training on sarcoplasmic reticulum Ca(2+) regulation in human skeletal muscle. *J. Appl. Physiol* 92, 912-922.

261. Liefeldt G., Noakes T.D., and Dennis S.C. (1992) Oxygen delivery does not limit peak running speed during incremental downhill running to exhaustion. *Eur. J. Appl. Physiol Occup. Physiol* 64, 493-496.

262. Lin R.Y., Vera J.C., Chaganti R.S., and Golde D.W. (1998) Human monocarboxylate transporter 2 (MCT2) is a high affinity pyruvate transporter. *J. Biol. Chem.* 273, 28959-28965.

263. Lindinger M.I. and Sjogaard G. (1991) Potassium regulation during exercise and recovery. *Sports Med.* 11, 382-401.

264. Linssen W.H., Jacobs M., Stegeman D.F., Joosten E.M., and Moleman J. (1990) Muscle fatigue in McArdle's disease. Muscle fibre conduction velocity and surface EMG frequency spectrum during ischaemic exercise. *Brain* 113 (Pt 6), 1779-1793.

265. Lloyd A.R., Gandevia S.C., and Hales J.P. (1991) Muscle performance, voluntary activation, twitch properties and perceived effort in normal subjects and patients with the chronic fatigue syndrome. *Brain* 114 (Pt 1A), 85-98.

266. Lloyd A.R. and Pender H. (1992) The economic impact of chronic fatigue syndrome. *Med. J. Aust.* 157, 599-601.

267. Lo Conte L.R. and Merletti R. (1996) Estimating EMG spectral compression: comparison of four indices. *Proc. 8th Ann. Con. IEEE Eng. Med. Biol. Sci.* 5, 2-5.

268. Lombard W.P. (1892) Some of the influences which affect the power of voluntary muscular contractions. *J. Physiol* 13, 1-58.

269. Lopes da Silva F.H. (1992) The rhythmic slow activity (theta) of the limbic cortex: an oscillation in search of a function. In Induced Rhythms in the Brain (ed. Basar E. and Bullock T.H.), pp. 269-283. Birkhäuser, Boston.

270. Lopes da Silva F.H., Witter M.P., Boeijinga P.H., and Lohman A.H. (1990) Anatomic organization and physiology of the limbic cortex. *Physiol Rev.* 70, 453-511.

271. Lorentzon R., Johansson C., Sjostrom M., Fagerlund M., and Fugl-Meyer A.R. (1988) Fatigue during dynamic muscle contractions in male sprinters and marathon runners: relationships between performance, electromyographic activity, muscle cross-sectional area and morphology. *Acta Physiol Scand.* 132, 531-536.

272. Lortie G., Simoneau J.A., Hamel P., Boulay M.R., and Bouchard C. (1985) Relationships between skeletal muscle characteristics and aerobic performance in sedentary and active subjects. *Eur. J. Appl. Physiol Occup. Physiol* 54, 471-475.

273. Loscher W.N., Cresswell A.G., and Thorstensson A. (1994) Electromyographic responses of the human triceps surae and force tremor during sustained submaximal isometric plantar flexion. *Acta Physiol Scand.* 152, 73-82.

274. Loscher W.N., Cresswell A.G., and Thorstensson A. (1996) Central fatigue during a long-lasting submaximal contraction of the triceps surae. *Exp. Brain Res.* 108, 305-314.

275. Loscher W.N., Cresswell A.G., and Thorstensson A. (1996) Excitatory drive to the alpha-motoneuron pool during a fatiguing submaximal contraction in man. *J. Physiol* 491 (Pt 1), 271-280.

276. Lowery M., Nolan P., and O'Malley M. (2002) Electromyogram median frequency, spectral compression and muscle fibre conduction velocity during sustained sub-maximal contraction of the brachioradialis muscle. *J. Electromyogr. Kinesiol.* 12, 111-118.

277. Lowery M., O'Malley M., Vaughan C., and St Clair Gibson A. (1998) A physiologically based simulation of the electromyographic signal. *Proc. 12th Ann. Con. IEEE Eng. Med. Biol. Sci.* 5, 12-13.

278. Loze G.M., Collins D., and Shaw J.C. (1999) EEG alpha rhythm, intention and oculomotor control. *Int. J. Psychophysiol.* 33, 163-167.

279. Luscher H.R., Ruenzel P., and Henneman E. (1979) How the size of motoneurones determines their susceptibility to discharge. *Nature* 282, 859-861.

280. Macefield G., Hagbarth K.E., Gorman R., Gandevia S.C., and Burke D. (1991) Decline in spindle support to alpha-motoneurones during sustained voluntary contractions. *J. Physiol* 440, 497-512.

281. Mallie J.P., Ait-Djafer Z., Saunders C., Pierrat A., Caira M.V., Courroy O., Panescu V., and Perrin P. (2002) Renal handling of salt and water in humans during exercise with or without hydration. *Eur. J. Appl. Physiol* 86, 196-202.

282. Manning Fox J.E., Meredith D., and Halestrap A.P. (2000) Characterisation of human monocarboxylate transporter 4 substantiates its role in lactic acid efflux from skeletal muscle. *J. Physiol* 529 Pt 2, 285-293.

283. Marino F.E. (2002) Methods, advantages, and limitations of body cooling for exercise performance. *Br. J. Sports Med.* 36, 89-94.

284. Marino F.E., Lambert M.I., and Noakes T.D. (2003) SUPERIOR PERFORMANCE OF AFRICAN RUNNERS IN WARM HUMID BUT NOT IN COOL ENVIRONMENTAL CONDITIONS. *J. Appl. Physiol.*

285. Marino F.E., Mbambo Z., Kortekaas E., Wilson G., Lambert M.I., Noakes T.D., and Dennis S.C. (2000) Advantages of smaller body mass during distance running in warm, humid environments. *Pflugers Arch.* 441, 359-367.

286. Marino F.E., Mbambo Z., Kortekaas E., Wilson G., Lambert M.I., Noakes T.D., and Dennis S.C. (2001) Influence of ambient temperature on plasma ammonia and lactate accumulation during prolonged submaximal and self-paced running. *Eur. J. Appl. Physiol* 86, 71-78.

287. Marsden J.F., Ashby P., Limousin-Dowsey P., Rothwell J.C., and Brown P. (2000) Coherence between cerebellar thalamus, cortex and muscle in man: cerebellar thalamus interactions. *Brain* 123 (Pt 7), 1459-1470.

288. Martin A.D., Spenst L.F., Drinkwater D.T., and Clarys J.P. (1990) Anthropometric estimation of muscle mass in men. *Med. Sci. Sports Exerc.* 22, 729-733.

289. Mason P. and Wilkinson G. (1996) The prevalence of psychiatric morbidity. OPCS survey of psychiatric morbidity in Great Britain. *Br. J. Psychiatry* 168, 1-3.

290. Masuda K., Masuda T., Sadoyama T., Inaki M., and Katsuta S. (1999) Changes in surface EMG parameters during static and dynamic fatiguing contractions. *J. Electromyogr. Kinesiol.* 9, 39-46.

291. Matthews P.B. (1997) Spindle and motoneuronal contributions to the phase advance of the human stretch reflex and the reduction of tremor. *J. Physiol* 498 (Pt 1), 249-275.

292. Maughan R.J., Nimmo M.A., and Harmon M. (1985) The relationship between muscle myosin ATP-ase activity and isometric endurance in untrained male subjects. *Eur. J. Appl. Physiol Occup. Physiol* 54, 291-296.

293. McArdle W.D., Katch F.I., and Katch V.L. (2002) Measurement of human energy expenditure. In Exercise physiology Lippincott Williams and Wilkins, Baltimore.

294. McAuley J.H. and Marsden C.D. (2000) Physiological and pathological tremors and rhythmic central motor control. *Brain* 123 (Pt 8), 1545-1567.

295. McAuley J.H., Rothwell J.C., and Marsden C.D. (1997) Frequency peaks of tremor, muscle vibration and electromyographic activity at 10 Hz, 20 Hz and 40 Hz during human finger muscle contraction may reflect rhythmicities of central neural firing. *Exp. Brain Res.* 114, 525-541.

296. McClelland G.B. and Brooks G.A. (2002) Changes in MCT 1, MCT 4, and LDH expression are tissue specific in rats after long-term hypobaric hypoxia. *J. Appl. Physiol* 92, 1573-1584.

297. McClelland G.B., Khanna S., Gonzalez G.F., Butz C.E., and Brooks G.A. (2003) Peroxisomal membrane monocarboxylate transporters: evidence for a redox shuttle system? *Biochem. Biophys. Res. Commun.* 304, 130-135.

298. McCullagh K.J., Poole R.C., Halestrap A.P., O'Brien M., and Bonen A. (1996) Role of the lactate transporter (MCT1) in skeletal muscles. *Am. J. Physiol* 271, E143-E150.

299. McDermott J.C. and Bonen A. (1993) Endurance training increases skeletal muscle lactate transport. *Acta Physiol Scand.* 147, 323-327.

300. McKenna M.J. (1992) The roles of ionic processes in muscular fatigue during intense exercise. *Sports Med.* 13, 134-145.

301. McKenna M.J. (1995) Effects of training on potassium homeostasis during exercise. *J. Mol. Cell Cardiol.* 27, 941-949.

302. McKenna M.J., Harmer A.R., Fraser S.F., and Li J.L. (1996) Effects of training on potassium, calcium and hydrogen ion regulation in skeletal muscle and blood during exercise. *Acta Physiol Scand.* 156, 335-346.

303. McKenna M.J., Heigenhauser G.J., McKelvie R.S., MacDougall J.D., and Jones N.L. (1997) Sprint training enhances ionic regulation during intense exercise in men. *J. Physiol* 501 (Pt 3), 687-702.

304. McKenna M.J., Schmidt T.A., Hargreaves M., Cameron L., Skinner S.L., and Kjeldsen K. (1993) Sprint training increases human skeletal muscle Na(+)-K(+)-ATPase concentration and improves K+ regulation. *J. Appl. Physiol* 75, 173-180.

305. Mechau D., Mucke S., Weiss M., and Liesen H. (1998) Effect of increasing running velocity on electroencephalogram in a field test. *Eur. J. Appl. Physiol Occup. Physiol* 78, 340-345.

306. Medbo J.I. and Sejersted O.M. (1990) Plasma potassium changes with high intensity exercise. *J. Physiol* 421, 105-122.

307. Merezhinskaya N., Fishbein W.N., Davis J.I., and Foellmer J.W. (2000) Mutations in MCT1 cDNA in patients with symptomatic deficiency in lactate transport. *Muscle Nerve* 23, 90-97.

308. Merletti R. and Lo Conte L.R. (1997) Surface EMG signal processing during isometric contractions. *J. Electromyogr. Kinesiol.* 7, 241-250.

309. Mero A., Jaakkola L., and Komi P.V. (1991) Relationships between muscle fibre characteristics and physical performance capacity in trained athletic boys. *J. Sports Sci.* 9, 161-171.

310. Miller E.K. (2000) The prefrontal cortex and cognitive control. *Nat. Rev. Neurosci.* 1, 59-65.

311. Miller R. (1991) Cotrico-hippocampal interplay and the representation of contexts in the brain. Springer, Berlin.

312. Miller R.G., Kent-Braun J.A., Sharma K.R., and Weiner M.W. (1995) Mechanisms of human muscle fatigue. Quantitating the contribution of metabolic factors and activation impairment. *Adv. Exp. Med. Biol.* 384, 195-210.

313. Mima T., Matsuoka T., and Hallett M. (2001) Information flow from the sensorimotor cortex to muscle in humans. *Clin. Neurophysiol.* 112, 122-126.

314. Mima T., Simpkins N., Oluwatimilehin T., and Hallett M. (1999) Force level modulates human cortical oscillatory activities. *Neurosci. Lett.* 275, 77-80.

315. Mima T., Steger J., Schulman A.E., Gerloff C., and Hallett M. (2000) Electroencephalographic measurement of motor cortex control of muscle activity in humans. *Clin. Neurophysiol.* 111, 326-337.

316. Miyamura M., Ishida K., Ohkuwa T., and Itoh H. (1994) Relationship between maximal pulmonary ventilation during exhaustive exercise and postexercise plasma potassium concentration in man. *Jpn. J. Physiol* 44, 117-122.

317. Mole P.A. and Hoffmann J.J. (1999) VO(2) kinetics of mild exercise are altered by RER. *J. Appl. Physiol* 87, 2097-2106.

318. Montain S.J. and Coyle E.F. (1992) Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *J. Appl. Physiol* 73, 1340-1350.

319. Morgan D.W., Bransford D.R., Costill D.L., Daniels J.T., Howley E.T., and Krahenbuhl G.S. (1995) Variation in the aerobic demand of running among trained and untrained subjects. *Med. Sci. Sports Exerc.* 27, 404-409.

320. Moritani T. and Muro M. (1987) Motor unit activity and surface electromyogram power spectrum during increasing force of contraction. *Eur. J. Appl. Physiol Occup. Physiol* 56, 260-265.

321. Moritani T., Muro M., and Kijima A. (1985) Electromechanical changes during electrically induced and maximal voluntary contractions: electrophysiologic responses of different muscle fiber types during stimulated contractions. *Exp. Neurol.* 88, 471-483.

322. Moritani T., Muro M., Kijima A., Gaffney F.A., and Parsons D. (1985) Electromechanical changes during electrically induced and maximal voluntary contractions: surface and intramuscular EMG responses during sustained maximal voluntary contraction. *Exp. Neurol.* 88, 484-499.

323. Moritani T. and Yoshitake Y. (1998) 1998 ISEK Congress Keynote Lecture: The use of electromyography in applied physiology. International Society of Electrophysiology and Kinesiology. *J. Electromyogr. Kinesiol.* 8, 363-381.

324. Mosso A. (1904) Fatigue. Swan Sonnenschein, London.

325. Mullany H. POO. [2.2]. 2000. (Computer Program)

326. Mullany H. MUPPET. 2001. (Computer Program)

327. Mullany H. and Myers L.J. FRAGGLE7. 2002. (Computer Program)

328. Mullany H., O'Malley M., St Clair G.A., and Vaughan C. (2002) Agonist-antagonist common drive during fatiguing knee extension efforts using surface electromyography. *J. Electromyogr. Kinesiol.* 12, 375-384.

329. Myburgh K.H. and Weston A.R. (1998) The human endurance athlete: heterogeneity and adaptability of selected exercise and skeletal muscle characteristics. *S. Afr. J. Zool.* 33, 129-140.

330. Nakae Y., Stoward P.J., Shono M., and Matsuzaki T. (1999) Localisation and quantification of dehydrogenase activities in single muscle fibers of mdx gastrocnemius. *Histochem. Cell Biol.* 112, 427-436.

331. Nielsen B., Hyldig T., Bidstrup F., Gonzalez-Alonso J., and Christoffersen G.R. (2001) Brain activity and fatigue during prolonged exercise in the heat. *Pflugers Arch.* 442, 41-48.

332. Nielsen O.B., de Paoli F., and Overgaard K. (2001) Protective effects of lactic acid on force production in rat skeletal muscle. *J. Physiol* 536, 161-166.

333. Nikolaev A.R., Ivanitsky G.A., Ivanitsky A.M., Posner M.I., and Abdullaev Y.G. (2001) Correlation of brain rhythms between frontal and left temporal (Wernicke's) cortical areas during verbal thinking. *Neurosci. Lett.* 298, 107-110.

334. Nindl B.C., Kraemer W.J., Emmert W.H., Mazzetti S.A., Gotshalk L.A., Putukian M., Sebastianelli W.J., and Patton J.F. (1998) Comparison of body composition assessment among lean black and white male collegiate athletes. *Med. Sci. Sports Exerc.* 30, 769-776.

335. Noakes T.D. (1988) Implications of exercise testing for prediction of athletic performance: a contemporary perspective. *Med. Sci. Sports Exerc.* 20, 319-330.

336. Noakes T.D. (1997) 1996 J.B. Wolffe Memorial Lecture. Challenging beliefs: ex Africa semper aliquid novi. *Med. Sci. Sports Exerc.* 29, 571-590.

337. Noakes T.D. (1998) Maximal oxygen uptake: "classical" versus "contemporary" viewpoints: a rebuttal. *Med. Sci. Sports Exerc.* 30, 1381-1398.

338. Noakes T.D. (1998) Why do Africans run so swiftly? A research challenge for African scientists. *South African Journal of Science* 94, 531-535.

339. Noakes T.D., Myburgh K.H., and Schall R. (1990) Peak treadmill running velocity during the VO2 max test predicts running performance. *J. Sports Sci.* 8, 35-45.

340. Noakes T.D., Peltonen J.E., and Rusko H.K. (2001) Evidence that a central governor regulates exercise performance during acute hypoxia and hyperoxia. *J. Exp. Biol.* 204, 3225-3234.

341. Noakes T.D., St Clair G.A., and Lambert E.V. (2004) From catastrophe to complexity: a novel model of integrative central neural regulation of effort and fatigue during exercise in humans. *Br. J. Sports Med.* 38, 511-514.

342. Nose H., Mack G.W., Shi X.R., and Nadel E.R. (1988) Shift in body fluid compartments after dehydration in humans. *J. Appl. Physiol* 65, 318-324.

343. Nybo L. (2003) CNS Fatigue and Prolonged Exercise: Effect of Glucose Supplementation. *Med. Sci. Sports Exerc.* 35, 589-594.

344. Nybo L. and Nielsen B. (2001) Hyperthermia and central fatigue during prolonged exercise in humans. *J. Appl. Physiol* 91, 1055-1060.

345. Nybo L. and Nielsen B. (2001) Perceived exertion is associated with an altered brain activity during exercise with progressive hyperthermia. *J. Appl. Physiol* 91, 2017-2023.

346. Oda S. and Kida N. (2001) Neuromuscular fatigue during maximal concurrent hand grip and elbow flexion or extension. *J. Electromyogr. Kinesiol.* 11, 281-289.

347. Olsen H.B., Christensen H., and Sogaard K. (2001) An analysis of motor unit firing pattern during sustained low force contraction in fatigued muscle. *Acta Physiol Pharmacol. Bulg.* 26, 73-78.

348. Orlander J., Kiessling K.H., and Ekblom B. (1980) Time course of adaptation to low intensity training in sedentary men: dissociation of central and local effects. *Acta Physiol Scand.* 108, 85-90.

349. Paavolainen L., Nummela A., Rusko H., and Hakkinen K. (1999) Neuromuscular characteristics and fatigue during 10 km running. *Int. J. Sports Med.* 20, 516-521.

350. Paavolainen L.M., Nummela A.T., and Rusko H.K. (1999) Neuromuscular characteristics and muscle power as determinants of 5-km running performance. *Med. Sci. Sports Exerc.* 31, 124-130.

351. Parkin S., Nowicky A.V., Rutherford O.M., and McGregor A.H. (2001) Do oarsmen have asymmetries in the strength of their back and leg muscles? *J. Sports Sci.* 19, 521-526.

352. Paulesu E., Frith C.D., and Frackowiak R.S. (1993) The neural correlates of the verbal component of working memory. *Nature* 362, 342-345.

353. Pellerin L., Pellegri G., Bittar P.G., Charnay Y., Bouras C., Martin J.L., Stella N., and Magistretti P.J. (1998) Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. *Dev. Neurosci.* 20, 291-299.

354. Petruzzello S.J. and Landers D.M. (1994) State anxiety reduction and exercise: does hemispheric activation reflect such changes? *Med. Sci. Sports Exerc.* 26, 1028-1035.

355. Pette D. and Staron R.S. (2000) Myosin isoforms, muscle fiber types, and transitions. *Microsc. Res. Tech.* 50, 500-509.

356. Pette D. and Vrbova G. (1985) Neural control of phenotypic expression in mammalian muscle fibers. *Muscle Nerve* 8, 676-689.

357. Pfurtscheller G. and Lopes da Silva F.H. (1999) Event-related EEG/MEG synchronization and desynchronization: basic principles. *Clin. Neurophysiol.* 110, 1842-1857.

358. Philp N.J., Yoon H., and Grollman E.F. (1998) Monocarboxylate transporter MCT1 is located in the apical membrane and MCT3 in the basal membrane of rat RPE. *Am. J. Physiol* 274, R1824-R1828.

359. Pilegaard H., Bangsbo J., Richter E.A., and Juel C. (1994) Lactate transport studied in sarcolemmal giant vesicles from human muscle biopsies: relation to training status. *J. Appl. Physiol* 77, 1858-1862.

360. Pilegaard H., Domino K., Noland T., Juel C., Hellsten Y., Halestrap A.P., and Bangsbo J. (1999) Effect of high-intensity exercise training on lactate/H+ transport capacity in human skeletal muscle. *Am. J. Physiol* 276, E255-E261.

361. Pilegaard H., Terzis G., Halestrap A., and Juel C. (1999) Distribution of the lactate/H+ transporter isoforms MCT1 and MCT4 in human skeletal muscle. *Am. J. Physiol* 276, E843-E848.

362. Pivarnik J.M., Bray M.S., Hergenroeder A.C., Hill R.B., and Wong W.W. (1995) Ethnicity affects aerobic fitness in US adolescent girls. *Med. Sci. Sports Exerc.* 27, 1635-1638.

363. Poole R.C. and Halestrap A.P. (1993) Transport of lactate and other monocarboxylates across mammalian plasma membranes. *Am. J. Physiol* 264, C761-C782.

364. Poole R.C., Sansom C.E., and Halestrap A.P. (1996) Studies of the membrane topology of the rat erythrocyte H+/lactate cotransporter (MCT1). *Biochem. J.* 320 (Pt 3), 817-824.

365. Popivanov D., Mineva A., and Krekule I. (1999) EEG patterns in theta and gamma frequency range and their probable relation to human voluntary movement organization. *Neurosci. Lett.* 267, 5-8.

366. Poso A.R. (2002) Monocarboxylate transporters and lactate metabolism in equine athletes: a review. *Acta Vet. Scand.* 43, 63-74.

367. Price N.T., Jackson V.N., and Halestrap A.P. (1998) Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. *Biochem. J.* 329 (Pt 2), 321-328.

368. Raine M.G. Anthro. [1]. 2001. (Computer Program)

369. Rainoldi A., Galardi G., Maderna L., Comi G., Lo C.L., and Merletti R. (1999) Repeatability of surface EMG variables during voluntary isometric contractions of the biceps brachii muscle. *J. Electromyogr. Kinesiol.* 9, 105-119.

370. Ray W.J. and Cole H.W. (1985) EEG alpha activity reflects attentional demands, and beta activity reflects emotional and cognitive processes. *Science* 228, 750-752.

371. Rearick M.P., Johnston J.A., and Slobounov S.M. (2001) Feedback-dependent modulation of isometric force control: an EEG study in visuomotor integration. *Brain Res. Cogn Brain Res.* 12, 117-130.

372. Rennie M.J. (2000) When having the nerve is helped by having the muscle. *J. Physiol* 522 Pt 1, 1.

373. Richter E.A., Kiens B., Saltin B., Christensen N.J., and Savard G. (1988) Skeletal muscle glucose uptake during dynamic exercise in humans: role of muscle mass. *Am. J. Physiol* 254, E555-E561.

374. Rodriguez Moreno D., Kim K.H.S., and Hirsch J. Verbal and object reasoning share some common brain regions: a functional magnetic resonance imaging (fMRI) study. Neuroimage. 13[6], S1312. 2001.

375. Ross W.D. and Marfell-Jones M.J. (1991) Kinanthropometry. In Physiological testing of the high-performance athlete (ed. MacDougall J.D., Wegner H.A., and Green H.S.), pp. 223-308. Human Kinetics Books, Champaign.

376. Roth D.A. and Brooks G.A. (1990) Lactate and pyruvate transport is dominated by a pH gradient-sensitive carrier in rat skeletal muscle sarcolemmal vesicles. *Arch. Biochem. Biophys.* 279, 386-394.

377. Roth D.A. and Brooks G.A. (1990) Lactate transport is mediated by a membrane-bound carrier in rat skeletal muscle sarcolemmal vesicles. *Arch. Biochem. Biophys.* 279, 377-385.

378. Rotto D.M. and Kaufman M.P. (1988) Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. *J. Appl. Physiol* 64, 2306-2313.

379. Saboisky J., Marino F.E., Kay D., and Cannon J. (2003) Exercise heat stress does not reduce central activation to non-exercised human skeletal muscle. *Exp. Physiol* 88, 783-790.

380. Sadoyama T. and Masuda T. (1987) Changes of the average muscle fiber conduction velocity during a varying force contraction. *Electroencephalogr. Clin. Neurophysiol.* 67, 495-497.

381. Sadoyama T. and Miyano H. (1981) Frequency analysis of surface EMG to evaluation of muscle fatigue. *Eur. J. Appl. Physiol Occup. Physiol* 47, 239-246.

382. Sahlin K. (1986) Muscle fatigue and lactic acid accumulation. *Acta Physiol Scand. Suppl* 556, 83-91.

383. Salmons S. (1994) Exercise, stimulation and type transformation of skeletal muscle. *Int. J. Sports Med.* 15, 136-141.

384. Saltin B. (2003) The Kenya Project - Final Report. *New Studies in Athletics* 18, 15-24.

385. Saltin B. and Astrand P.O. (1967) Maximal oxygen uptake in athletes. *J. Appl. Physiol* 23, 353-358.

386. Saltin B., Henriksson J., Nygaard E., Andersen P., and Jansson E. (1977) Fiber types and metabolic potentials of skeletal muscles in sedentary man and endurance runners. *Ann. N. Y. Acad. Sci.* 301, 3-29.

387. Saltin B., Kim C.K., Terrados N., Larsen H., Svedenhag J., and Rolf C.J. (1995) Morphology, enzyme activities and buffer capacity in leg muscles of Kenyan and Scandinavian runners. *Scand. J. Med. Sci. Sports* 5, 222-230.

388. Saltin B., Larsen H., Terrados N., Bangsbo J., Bak T., Kim C.K., Svedenhag J., and Rolf C.J. (1995) Aerobic exercise capacity at sea level and at altitude in Kenyan boys, junior and senior runners compared with Scandinavian runners. *Scand. J. Med. Sci. Sports* 5, 209-221.

389. Saltin B. and Strange S. (1992) Maximal oxygen uptake: "old" and "new" arguments for a cardiovascular limitation. *Med. Sci. Sports Exerc.* 24, 30-37.

390. Saper C.B. (1982) Convergence of autonomic and limbic connections in the insular cortex of the rat. *J. Comp Neurol.* 210, 163-173.

391. Sauseng P., Klimesch W., Gruber W., Doppelmayr M., Stadler W., and Schabus M. (2002) The interplay between theta and alpha oscillations in the human electroencephalogram reflects the transfer of information between memory systems. *Neurosci. Lett.* 324, 121-124.

392. Schantz P.G. and Dhoot G.K. (1987) Coexistence of slow and fast isoforms of contractile and regulatory proteins in human skeletal muscle fibres induced by endurance training. *Acta Physiol Scand.* 131, 147-154.

393. Schanze T. and Eckhorn R. (1997) Phase correlation among rhythms present at different frequencies: spectral methods, application to microelectrode recordings from visual cortex and functional implications. *Int. J. Psychophysiol.* 26, 171-189.

394. Schiaffino S. and Salviati G. (1997) Molecular diversity of myofibrillar proteins: isoforms analysis at the protein and mRNA level. *Methods Cell Biol.* 52, 349-369.

395. Schillings M.L., Hoefsloot W., Stegeman D.F., and Zwarts M.J. (2003) Relative contributions of central and peripheral factors to fatigue during a maximal sustained effort. *Eur. J. Appl. Physiol* 90, 562-568.

396. Schurmann M. and Basar E. (2001) Functional aspects of alpha oscillations in the EEG. *Int. J. Psychophysiol.* 39, 151-158.

397. Schutte J.E., Townsend E.J., Hugg J., Shoup R.F., Malina R.M., and Blomqvist C.G. (1984) Density of lean body mass is greater in blacks than in whites. *J. Appl. Physiol* 56, 1647-1649.

398. Schwartz A.L. (1999) Fatigue mediates the effects of exercise on quality of life. *Qual. Life Res.* 8, 529-538.

399. Scott B.K. and Houmard J.A. (1994) Peak running velocity is highly related to distance running performance. *Int. J. Sports Med.* 15, 504-507.

400. Sepponen K., Koho N., Puolanne E., Ruusunen M., and Poso A.R. (2003) Distribution of monocarboxylate transporter isoforms MCT1, MCT2 and MCT4 in porcine muscles. *Acta Physiol Scand.* 177, 79-86.

401. Sharwood K.A. The effects of endurance training on neuromuscular characteristics in masters runners. 2003. University of Cape Town. PhD Thesis.

402. Shaw J.C. (1996) Intention as a component of the alpha-rhythm response to mental activity. *Int. J. Psychophysiol.* 24, 7-23.

403. Shulman R.G. and Rothman D.L. (2001) The "glycogen shunt" in exercising muscle: A role for glycogen in muscle energetics and fatigue. *Proc. Natl. Acad. Sci. U. S. A* 98, 457-461.

404. Simoneau J.A. and Bouchard C. (1995) Genetic determinism of fiber type proportion in human skeletal muscle. *FASEB J.* 9, 1091-1095.

405. Simoneau J.A., Lortie G., Boulay M.R., Marcotte M., Thibault M.C., and Bouchard C. (1985) Human skeletal muscle fiber type alteration with high-intensity intermittent training. *Eur. J. Appl. Physiol Occup. Physiol* 54, 250-253.

406. Singer T., Seymour B., O'Doherty J., Kaube H., Dolan R.J., and Frith C.D. (2004) Empathy for pain involves the affective but not sensory components of pain. *Science* 303, 1157-1162.

407. Sjogaard G. (1988) Muscle energy metabolism and electrolyte shifts during low-level prolonged static contraction in man. *Acta Physiol Scand.* 134, 181-187.

408. Sjogaard G. (1990) Exercise-induced muscle fatigue: the significance of potassium. *Acta Physiol Scand. Suppl* 593, 1-63.

409. Smith L.L. (2000) Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med. Sci. Sports Exerc.* 32, 317-331.

410. Speedy D.B., Noakes T.D., Kimber N.E., Rogers I.R., Thompson J.M., Boswell D.R., Ross J.J., Campbell R.G., Gallagher P.G., and Kuttner J.A. (2001) Fluid balance during and after an ironman triathlon. *Clin. J. Sport Med.* 11, 44-50.

411. St Clair Gibson A., Lambert E.V., Lambert M.I., Hampson D.B., and Noakes T.D. (2001) Exercise and fatigue-control mechanisms. *Int. SportMed. J.* 2.

412. St Clair Gibson A. and Noakes T.D. Evidence for complex system integration and dynamic neural regulation of skeletal muscle recruitment during exercise in humans. Br.J.Sports Med. 2004. (In Press)

413. St Clair Gibson A., Schabort E.J., and Noakes T.D. (2001) Reduced neuromuscular activity and force generation during prolonged cycling. *Am. J. Physiol Regul. Integr. Comp Physiol* 281, R187-R196.

414. St Clair Gibson A., Baden D.A., Lambert M.I., Lambert E.V., Harley Y.X., Hampson D., Russell V.A., and Noakes T.D. (2003) The conscious perception of the sensation of fatigue. *Sports Med.* 33, 167-176.

415. Stainsby W.N. and Brooks G.A. (1990) Control of lactic acid metabolism in contracting muscles and during exercise. *Exerc. Sport Sci. Rev.* 18, 29-63.

416. Stallknecht B., Vissing J., and Galbo H. (1998) Lactate production and clearance in exercise. Effects of training. A mini-review. *Scand. J. Med. Sci. Sports* 8, 127-131.

417. Stanley W.C., Gertz E.W., Wisneski J.A., Morris D.L., Neese R.A., and Brooks G.A. (1985) Systemic lactate kinetics during graded exercise in man. *Am. J. Physiol* 249, E595-E602.

418. Stanley W.C., Gertz E.W., Wisneski J.A., Neese R.A., Morris D.L., and Brooks G.A. (1986) Lactate extraction during net lactate release in legs of humans during exercise. *J. Appl. Physiol* 60, 1116-1120.

419. Stock C., Baum M., Rosskopf P., Schober F., Weiss M., and Liesen H. (1996) Electroencephalogram activity, catecholamines, and lymphocyte subpopulations after resistance exercise and during regeneration. *Eur. J. Appl. Physiol Occup. Physiol* 72, 235-241.

420. Stokes M.J., Cooper R.G., and Edwards R.H. (1988) Normal muscle strength and fatigability in patients with effort syndromes. *BMJ* 297, 1014-1017.

421. Stumbo T.A., Merriam S., Nies K., Smith A., Spurgeon D., and Weir J.P. (2001) The effect of hand-grip stabilization on isokinetic torque at the knee. *J. Strength. Cond. Res.* 15, 372-377.

422. Suchan B., Yaguez L., Wunderlich G., Canavan A.G., Herzog H., Tellmann L., Homberg V., and Seitz R.J. (2002) Hemispheric dissociation of visual-pattern processing and visual rotation. *Behav. Brain Res.* 136, 533-544.

423. Suzuki H., Conwit R.A., Stashuk D., Santarsiero L., and Metter E.J. (2002) Relationships between surface-detected EMG signals and motor unit activation. *Med. Sci. Sports Exerc.* 34, 1509-1517.

424. Svantesson U., Ernstoff B., Bergh P., and Grimby G. (1991) Use of a Kin-Com dynamometer to study the stretch-shortening cycle during plantar flexion. *Eur. J. Appl. Physiol Occup. Physiol* 62, 415-419.

425. Szczesna-Kaczmarek A. (1990) L-lactate oxidation by skeletal muscle mitochondria. *Int. J. Biochem.* 22, 617-620.

426. Talmadge R.J. and Roy R.R. (1993) Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. *J. Appl. Physiol* 75, 2337-2340.

427. Tanaka K. and Matsuura Y. (1982) A multivariate analysis of the role of certain anthropometric and physiological attributes in distance running. *Ann. Hum. Biol.* 9, 473-482.

428. Taylor A.D., Humphries B., Smith P., and Bronks R. (1997) Electrophoretic separation of myosin heavy chain isoforms in the human m. vastus lateralis: references to reproducibility and relationships with force, electromechanical delay, fibre conduction velocity, endurance and electromyography. *Arch. Physiol Biochem.* 105, 10-18.

429. Taylor J.L. and Gandevia S.C. (2001) Transcranial magnetic stimulation and human muscle fatigue. *Muscle Nerve* 24, 18-29.

430. Tergau F., Geese R., Bauer A., Baur S., Paulus W., and Reimers C.D. (2000) Motor cortex fatigue in sports measured by transcranial magnetic double stimulation. *Med. Sci. Sports Exerc.* 32, 1942-1948.

431. Termin A., Staron R.S., and Pette D. (1989) Myosin heavy chain isoforms in histochemically defined fiber types of rat muscle. *Histochemistry* 92, 453-457.

432. Theriault R., Boulay M.R., Theriault G., and Simoneau J.A. (1996) Electrical stimulation-induced changes in performance and fiber type proportion of human knee extensor muscles. *Eur. J. Appl. Physiol Occup. Physiol* 74, 311-317.

433. Thornton J.M., Guz A., Murphy K., Griffith A.R., Pedersen D.L., Kardos A., Leff A., Adams L., Casadei B., and Paterson D.J. (2001) Identification of higher brain centres that may encode the cardiorespiratory response to exercise in humans. *J. Physiol* 533, 823-836.

434. Thorstensson A. and Karlsson J. (1976) Fatiguability and fibre composition of human skeletal muscle. *Acta Physiol Scand.* 98, 318-322.

435. Tonouchi M., Hatta H., and Bonen A. (2002) Muscle contraction increases lactate transport while reducing sarcolemmal MCT4, but not MCT1. *Am. J. Physiol Endocrinol. Metab* 282, E1062-E1069.

436. Tucker R., Rauch L., Harley Y.X., and Noakes T.D. (2004) Impaired exercise performance in the heat is associated with an anticipatory reduction in skeletal muscle recruitment. *Pflugers Arch.* 448, 422-430.

437. Ulmer H.V. (1996) Concept of an extracellular regulation of muscular metabolic rate during heavy exercise in humans by psychophysiological feedback. *Experientia* 52, 416-420.

438. Viitasalo J.H. and Komi P.V. (1975) Signal characteristics of EMG with special reference to reproducibility of measurements. *Acta Physiol Scand.* 93, 531-539.

439. Viitasalo J.H. and Komi P.V. (1977) Signal characteristics of EMG during fatigue. *Eur. J. Appl. Physiol Occup. Physiol* 37, 111-121.

440. Viitasalo J.T. and Bosco C. (1982) Electromechanical behaviour of human muscles in vertical jumps. *Eur. J. Appl. Physiol Occup. Physiol* 48, 253-261.

441. Vissing J. (2000) Muscle reflex and central motor control of neuroendocrine activity, glucose homeostasis and circulation during exercise. *Acta Physiol Scand. Suppl* 647, 1-26.

442. Wallinga-De Jonge W., Gielen F.L., Wirtz P., De Jong P., and Broenink J. (1985) The different intracellular action potentials of fast and slow muscle fibres. *Electroencephalogr. Clin. Neurophysiol.* 60, 539-547.

443. Weir J.B. (1990) New methods for calculating metabolic rate with special reference to protein metabolism. 1949. *Nutrition* 6, 213-221.

444. Weltan S.M., Bosch A.N., Dennis S.C., and Noakes T.D. (1998) Influence of muscle glycogen content on metabolic regulation. *Am. J. Physiol* 274, E72-E82.

445. Wertheim A.H. (1981) Occipital alpha activity as a measure of retinal involvement in oculomotor control. *Psychophysiology* 18, 432-439.

446. West W., Hicks A., McKelvie R., and O'Brien J. (1996) The relationship between plasma potassium, muscle membrane excitability and force following quadriceps fatigue. *Pflugers Arch.* 432, 43-49.

447. Westgaard R.H. and De Luca C.J. (1999) Motor unit substitution in long-duration contractions of the human trapezius muscle. *J. Neurophysiol.* 82, 501-504.

448. Weston A.R., Karamizrak O., Smith A., Noakes T.D., and Myburgh K.H. (1999) African runners exhibit greater fatigue resistance, lower lactate accumulation, and higher oxidative enzyme activity. *J. Appl. Physiol* 86, 915-923.

449. Weston A.R., Mbambo Z., and Myburgh K.H. (2000) Running economy of African and Caucasian distance runners. *Med. Sci. Sports Exerc.* 32, 1130-1134.

450. Williamson J.W., McColl R., Mathews D., Ginsburg M., and Mitchell J.H. (1999) Activation of the insular cortex is affected by the intensity of exercise. *J. Appl. Physiol* 87, 1213-1219.

451. Williamson J.W., Nobrega A.C., McColl R., Mathews D., Winchester P., Friberg L., and Mitchell J.H. (1997) Activation of the insular cortex during dynamic exercise in humans. *J. Physiol* 503 (Pt 2), 277-283.

452. Wilson G.J., Elliott B.C., and Wood G.A. (1991) The effect on performance of imposing a delay during a stretch-shorten cycle movement. *Med. Sci. Sports Exerc.* 23, 364-370.

453. Wilson G.J., Wood G.A., and Elliott B.C. (1991) Optimal stiffness of series elastic component in a stretch-shorten cycle activity. *J. Appl. Physiol* 70, 825-833.

454. Wilson M.C., Jackson V.N., Heddle C., Price N.T., Pilegaard H., Juel C., Bonen A., Montgomery I., Hutter O.F., and Halestrap A.P. (1998) Lactic acid efflux from white skeletal muscle is catalyzed by the monocarboxylate transporter isoform MCT3. *J. Biol. Chem.* 273, 15920-15926.

455. Wilson M.C., Meredith D., and Halestrap A.P. (2002) Fluorescence resonance energy transfer studies on the interaction between the lactate transporter MCT1 and CD147 provide information on the topology and stoichiometry of the complex in situ. *J. Biol. Chem.* 277, 3666-3672.

456. Woods J.J., Furbush F., and Bigland-Ritchie B. (1987) Evidence for a fatigue-induced reflex inhibition of motoneuron firing rates. *J. Neurophysiol.* 58, 125-137.

457. Wyndham C.H., Strydom N.B., van Rensburg A.J., and Benade A.J. (1969) Physiological requirements for world-class performances in endurance running. *S. Afr. Med. J.* 43, 996-1002.

458. Yoon H., Fanelli A., Grollman E.F., and Philp N.J. (1997) Identification of a unique monocarboxylate transporter (MCT3) in retinal pigment epithelium. *Biochem. Biophys. Res. Commun.* 234, 90-94.

459. Zijdewind I., Zwarts M.J., and Kernell D. (1999) Fatigue-associated changes in the electromyogram of the human first dorsal interosseous muscle. *Muscle Nerve* 22, 1432-1436.

460. Zwarts M.J. and Arendt-Nielsen L. (1988) The influence of force and circulation on average muscle fibre conduction velocity during local muscle fatigue. *Eur. J. Appl. Physiol Occup. Physiol* 58, 278-283.